Energy Recovery Potential from Altona Treatment Plant and Meat Industry Wastes

A thesis submitted in fulfilment of the requirements for the degree of Master of Engineering

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DECLARATION

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

Yuanmei Sha

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ABSTRACT

City West Water (CWW) operates the Altona Treatment Plant (ATP), which treats predominantly domestic sewage. Increasing energy prices have shifted how wastewater treatment plants and other sludge generating industries perceive sludge. In the past, it was regarded as a disposal problem but now it is looked at as a feedstock for energy recovery. ATP utilises intermittent decanted extended aeration (IDEA) as a secondary treatment. The wasted activated sludge (WAS) is treated aerobically, and then the solids remaining are dewatered and are referred to as 'biosolids'. Every year, ATP produces 9,000 tons of biosolids containing 13% solids content, which is transported by subcontractors to offsite composting facilities. The expected growth in population in the area, meaning more biosolids will be produced, and the cost associated with sludge treatment, handling and transportation have triggered the ATP to seek alternative sludge and biosolids management. Therefore, one of the options CWW has decided to investigate is the energy recovery options from the sludge generated onsite, as well as other options for biosolids treatment onsite.

The first phase of this study comprised a literature review of alternative technologies for energy recovery from WAS and biosolids. The study identified pyrolysis, gasification, incineration and anaerobic digestion (AD) as alternative energy recovery processes. These alternatives were assessed based on the criteria of operation conditions, costs, energy input and output and economic factors. It was found that AD is the most feasible option for energy recovery from sludge in ATP.

The anaerobic digestion processes that were assessed as a part of this study comprised mesophilic AD (one phase) and temperature phased anaerobic digestion (TPAD). This study investigated biogas production from ATP's WAS and biosolids. The latter was used as a feedstock mainly to assess the degree of stabilisation of WAS achieved through the existing aerobic digestion process. Considering that there are constraints for the installation of anaerobic digestion at the ATP site, this study also assessed the co-digestion of WAS and meat processing dissolved air flotation (DAF) sludge using mesophilic AD and TPAD with a view to transporting biosolids to an alternative site. The performance of mesophilic AD and TPAD for WAS, biosolids and co-digestion of WAS and DAF was evaluated at different hydraulic retention times (HRT) and organic loading rates (OLR).

Biochemical methane potential (BMP) laboratory batch tests were used to estimate the biogas yield from the different substrates of WAS, biosolids and biosolids and DAF sludge. These tests showed that the biogas yield at an organic loading of 0.55-0.58 gCOD/gVS in descending order was 171, 145 and 129 mL/gVS, for WAS, biosolids and biosolids and DAF sludge, respectively, measured after 35 days. The biogas yield for WAS at 171 mL/gVS was in agreement with published results for IDEA sludge.

Given the promising results obtained using batch BMP tests, the performance of the mesophilic AD (one phase) and TPAD processes were assessed under semi-continuous conditions and different operation conditions of HRT and OLRs. The biogas yields from the different substrates, using mesophilic AD, were 282, 188, 180 and 86 mL/gVS from DAF sludge, WAS, biosolids and WAS and DAF sludge, respectively. Using TPAD, the biogas yields from different substrates were 235, 206 and 84 mL/gVS of WAS, biosolids and WAS and DAF sludge, respectively.

In terms of HRT, a typical relationship between the HRT and biogas yield was observed (e.g., biogas yield at a HRT of 23 days was higher than at a HRT of 14 days for all the substrates tested). Comparing the performance of the AD processes tested, using TPAD improved biogas yield from WAS and DAF sludge but no significant effect was observed for the substrates biosolids and WAS and DAF sludge.

The results demonstrated that, in comparison with other factors such as OLR and HRT, the type of waste was the factor that affected the AD performance the most. Overall, for the different substrates tested, biogas yield in descending order was as follows: WAS, biosolids, DAF sludge and WAS and DAF sludge.

The net energy recovery from the different substrates a both mesophilic AD and TPAD was estimated. To determine the net energy gain, the energy input for maintaining the temperature and mixing in the reactor were considered. The results showed that if ATP replaces the current aerobic sludge treatment with anaerobic sludge digestion, there will be savings on the energy used for sludge aeration. In light of the potential energy recovery analysis, the highest energy recovery option is mesophilic AD of WAS, which can save 2,266 MWh/yr from replacing the current aerobic system and also generate 465 MWh/yr from WAS methane yield (see Table 7-3). The energy generated from the methane gas can be converted to either thermal or electrical energy. Thus, based on results of the study, sludge from ATP can be a resource for energy recovery.

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- *Sha.Y,& Othman,M, 2012,'Effect Of Inoculum/Substrate Ratio On Mesophilic Anaerobic Digestion Of Waste-Activated Sludge In Batch Mode', OzWater 12 conference proceeding.*

- *Sha.Y,& Othman,M, 2013,' Anaerobic Digestion Of Was From Idea System Using Conventional And Temperature Phased Process', OzWater 13 . conference proceeding.*

LIST OF ABBREVIATIONS

- AD – Anaerobic digestion
- ATP – Altona Treatment Plant
- BMP – Biochemical Methane Potential (BMP)
- BNR – Biological Nitrogen Removal
- CODt – Total Chemical Oxygen Demand
- CODs – Soluble Chemical Oxygen Demand
- DAF – Dissolved Air Floatation
- FOG– Fat, Oil and Grease
- HRT – Hydraulic Retention Time
- IDEA – Intermittently Decanted Extended Aeration
- OLR – Organic Loading Rate
- pH – the negative logarithm of the hydrogen ion concentration
- SAN –Soluble Ammonia Nitrogen
- SRT – Sludge Retention Time, Solids Retention Time
- TAN –Total Ammonia Nitrogen
- TP – Total Phosphorous
- TN – Total Nitrogen
- TPAD – Temperature Phased anaerobic digestion (Two-phased anaerobic digestion)
- TS – Total Solids
- TSS – Total Suspended Solids
- TVFAs – Total Volatile Fatty Acids
- VS – Volatile Solids
- VSS – Volatile Suspended Solids
- WAS – Wasted Activated Sludge
- WWTPs– Wastewater Treatment Plants

1. General Introduction

1.1 Introduction

City West Water (CWW) operates Altona Treatment Plant (ATP), which treats predominantly domestic sewage. The ATP utilises a biological nitrogen removal process (BNR), which is an intermittent decant extended aeration (IDEA) system. The wasted activated sludge (WAS) produced is treated using an aerobic sludge digester, and the solids remaining after treatment are dewatered using two belt filter presses. The IDEA is operated at a sludge age of 10 to 14 days, whereas the aerobic sludge digestion reactors are operated at a sludge age of 18 days. The filter press usually produces biosolids of about 13% solids. The quantity of biosolids produced at ATP is around 8,000 to 9,000 tons/year. These biosolids are transported to an organics recycling facility, where they are mixed with council green waste for composting. The composted waste can be used as a soil conditioner and for landfill remediation.

Sludge and biosolids are comprised of different components. Some are useful (e.g., nutrients), but some are harmful, or need to be removed before they are transported or applied on land (e.g., heavy metals and pathogens). Land application was in many cases the most cost-effective alternative for sludge management (2004). However, the rising price and increased energy consumption led to an increase in the demand for the use of the sludge and/or biosolids as a source for its renewable energy recovery potential. There are a number of technologies available for energy recovery from sludge and biosolids (e.g., anaerobic digestion).

Waste from the meat industry, which contains plentiful carbon source, has good potential for codigestion with sewage sludge. However there maybe problems to cause failure of the digesters due to the nutrition balance, toxic chemicals, overloaded design, and so on. Hence, a scientific experimental approach was required to assess the best technical and environmental strategy.

1.2 Objectives and Scope

The aim of this project is to provide a summary of the current knowledge on energy recovery options from wastewater treatment plants sludge and/or biosolids. This covers technologies as well as data from case studies and research experimental results. The focus of the study is the ATP.

The project methodology comprised two stages. The first stage involved a review of the current literature. The objectives of this stage were to investigate alternative energy recovery technologies from sludge and biosolids at ATP, develop criteria for evaluation of these technologies and determine the most feasible technology for sludge management at ATP.

The second stage involved experimental work, which included characterisations of wastes being considered as a feedstock for anaerobic digestion, measurement of biochemical methane potential (BMP) and evaluation of different anaerobic digestion (AD) systems for the treatment of ATP sludge with and without mixing with other wastes.

The scope of the project comprised BMP for ATP's WAS and biosolids, and codigestion of ATP's sludge and biosolids with meat processing wastes. Feasibility of biogas production was assessed under batch conditions (i.e., BMP tests as well as semi-continuous conditions), using one phase AD and two-phase AD.

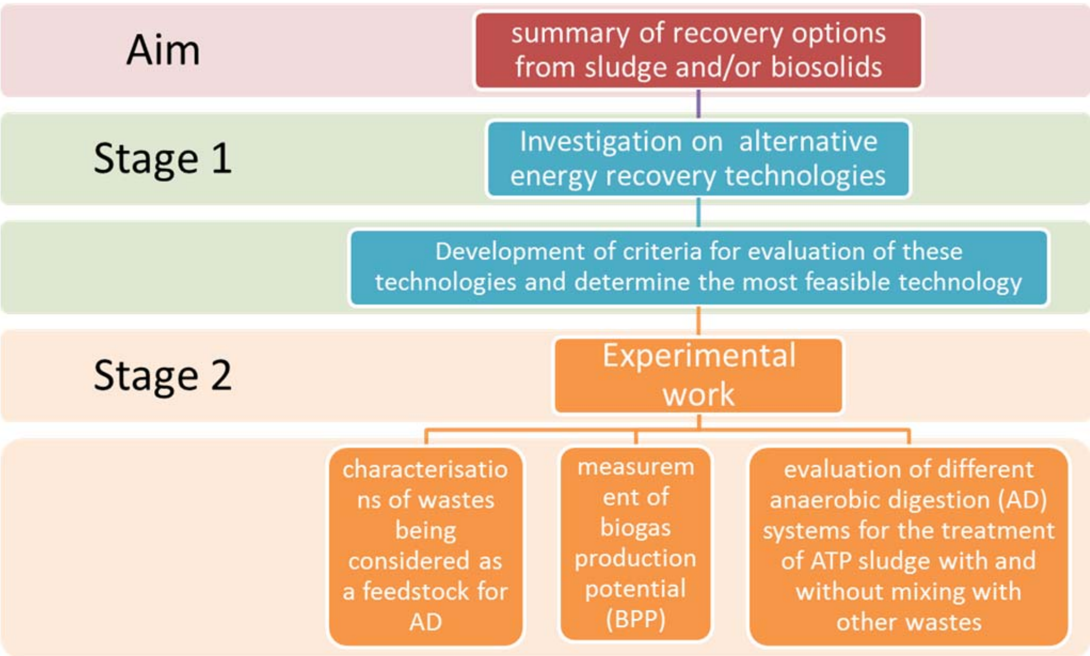

Figure 1-1 Scheme of objectives and scope

2. Background

2.1 Sludge Disposal Issue

WAS is the excess activated sludge that is generated as a by-product of the wastewater treatment process. WAS has a high level of pathogens; therefore, it has to be treated to reduce or eliminate its pathogen level to ensure that it poses no health or environmental risk for the intended beneficial use. The WAS after treatment is termed biosolids.

In Australia, the national biosolids production is about 0.36 M dry tons/year, whereas the US and the European Union each generate 7.5 M dry tons/year. In Victoria, the annual biosolids production was estimated to be 66,700 dry tons/year and only less than 5% have beneficial uses (Department of Natural Resources and Environment, 2002). The average cost for management of biosolids was estimated to be between $300-400 per dry ton (Table 2-1), which means Victorians spend around $20 M to manage biosolids annually.

Table 2-1: Costs for Sludge Disposal (Bolzonella et al., 2007)

Sludge Disposal	Disposal Costs, euros/ton
Agriculture	100–270
Landfilling	150–400
Incineration	300–600
Composting	150–400

Increasing energy prices shift the focus from regarding sludge as a disposal problem to utilising sludge as a substrate for energy recovery. Currently, every year ATP alone produces 9,000 tons of biosolids of 13% solids content, and then subcontractors transport the biosolids for compost.

2.2 Carbon Emission from Sludge

Proper sludge treatment also reduces greenhouse gas (GHG) emissions. Comparing waste discharge to landfills, energy recovery from waste can reduce GHG emissions through: (1) avoiding/reducing methane emissions from landfills and (2) reducing energy production from fossil fuel combustion, hence, reducing GHG emissions (Mata-Alvarez et al.,2000).

2.3 ATP

The focus of this project was the sludge and biosolids generated at ATP, managed and operated by CWW. ATP is located on 293 Queen Street, Altona Meadows, Victoria, about 20 km south west of Melbourne. ATP has a sewer catchment of 17.8 square kilometres and receives predominantly domestic sewage. It services around 50,000 in the suburbs of Altona, Altona Meadows, Laverton, Point Cook and Sanctuary Lakes.

2.3.1. Wastewater Process

The ATP's daily average wastewater flow is 12.5 ML/d. The wastewater process flowchart is shown on Figure 2-1 below.

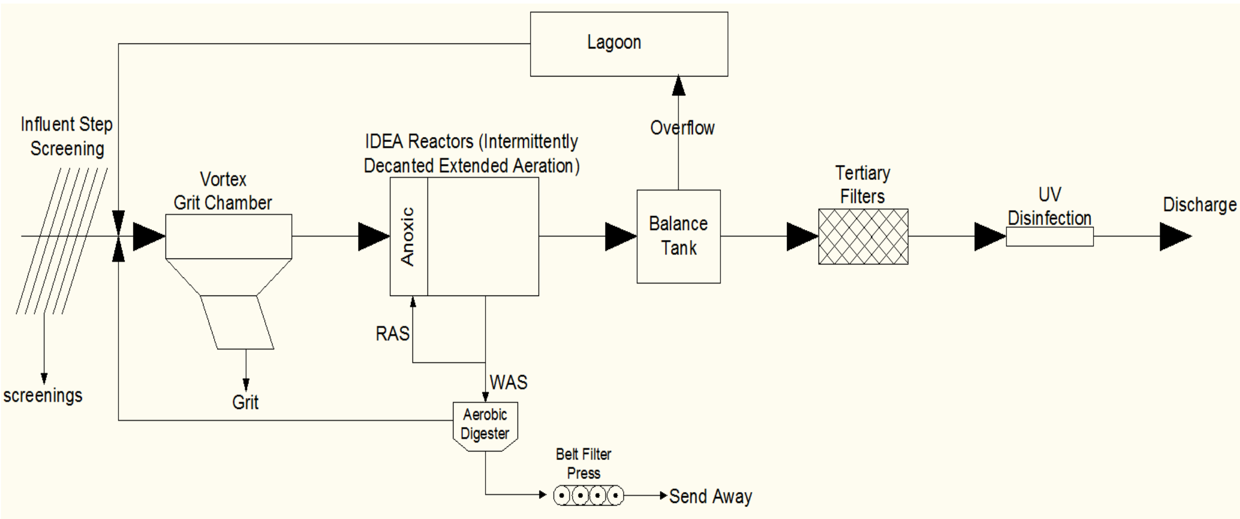

Figure 2-1: ATP wastewater treatment process flowchart

The wastewater at ATP passes through three IDEA reactors. Compared with conventional activate sludge, the extended aeration process operates at a sufficiently long sludge age (12 days in the case of the ATP) and low food to microorganism ratio of around 0.04 to 0.1 kgBOD5/kgTSS .day compared to 0.8 to 0.15 kgBOD/kgTSS .day in conventional activated sludge treatment processes. This results in less excess sludge production because the biomass yield depends on the sludge age (Foladori et al.,2010).

2.3.2. **Aerobic Sludge Treatment**

ATP currently applies aerobic digestion for the stabilisation and solids reduction of WAS produced from IDEA. The aerobic digester receives 1.3 ML WAS, approximately 4,375 kg solids/day. Sludge from the IDEA reactor is aerated, and the organic fraction is degraded for 18 days in the aerobic sludge digester. The digested sludge is mixed with a polymer, then sent to a belt filter presses where it is dewatered into biosolids. Aerobic sludge stabilisation process at ATP achieves 23% reduction in total solids on average.

The mass balance diagram of sludge in ATP is shown in Figure 2-2.

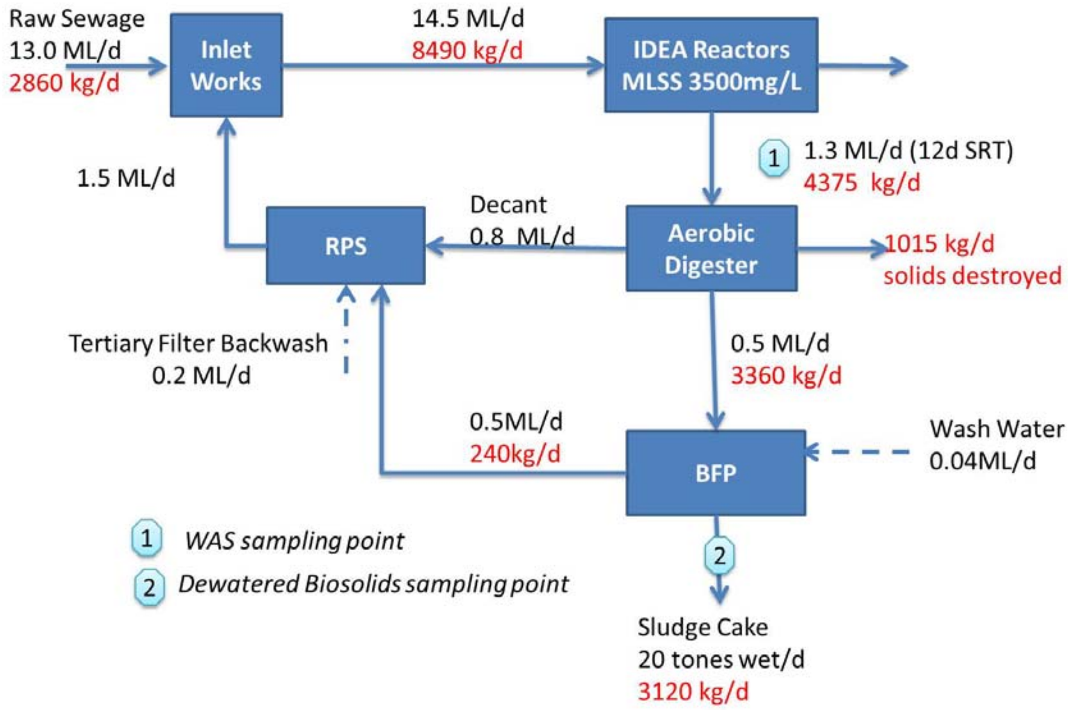

Figure 2-2: Sludge mass balance of ATP

Under the current waste management practice, the dewatered biosolids are transported to an organics recycling facility where it is mixed with council green waste for composting. The compost waste is ultimately used as a soil conditioner or for landfill remediation. In the meantime, CWW is looking into alternative sustainable sludge treatment options.

3. Sludge generalities

3.1 Sludge Characteristics

The influent of a wastewater treatment plant varies from day to day. This results in a variation in the characteristics of the sludge, which have a direct effect on its BMP. CWW arranges weekly tests of WAS and aerobic digested sludge and biosolids in ATP. Summary and analysis of the three-year data provided by CWW is shown in section 3.1.2 (July 2008 to April 2011).

3.1.1. **Sludge Composition and Heating Value**

The world average sludge composition indicates typical sludge elements (see Table 3-1). The heating value of raw sludge is about 17 MJ/kg, for activated sludge, about 15 MJ/kg, and for stabilised sludge (digested: anaerobic, aerobic or lime stabilisation) around 11 MJ/kg (Werle et al.,2010).

Table 3-1: Typical Sludge Composition (Paul et al.,2012)

Element wt %	Typical sludge World average
C	40.57
H	5.36
O	23.50
N	4.50
S	1.20
Cl	0.04
P	24.83
Heating value , MJ/kg	15.10

3.1.2. **WAS from ATP**

Sludge samples are collected from the inlet to the aerobic sludge digester, shown as sampling point 1 in Figure 2-2. To ensure the consistency, all WAS samples were collected from tank TA102, which is one of the three IDEA reactors.

The characteristics of WAS from ATP, pH, total suspended solids (TSS), volatile suspended solids (VSS) and alkalinity are shown in Table 3-2. The average VSS/TSS is over 80%, which indicates good potential for energy recovery and solids reduction through AD

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Table 3-2: Characteristics of WAS in ATP (Data from July 2009 to March 2011)

Parameters	pH	TSS	VSS	Alk-Total CaCO	%VSS/TSS
Unit		*mg/L*	*mg/L*	*mg $CaCO_3$/L*	*%*
Average	7.04	4056	3376	265.8	83.22
SD	0.302	768.6	636.4	37.45	4.325
Min	6.6	450	2800	200	52.94
Max	8.3	6200	5000	450	93.18

Considering that VSS is a critical parameter for energy recovery as it represents the organic fraction of the sludge, it is important to have a close look at the changes in VSS concentration overtime. As shown in Figure 3-1, there was a clear seasonal variation during the first year of data recording, but little variation in the characteristics was observed after 2009.

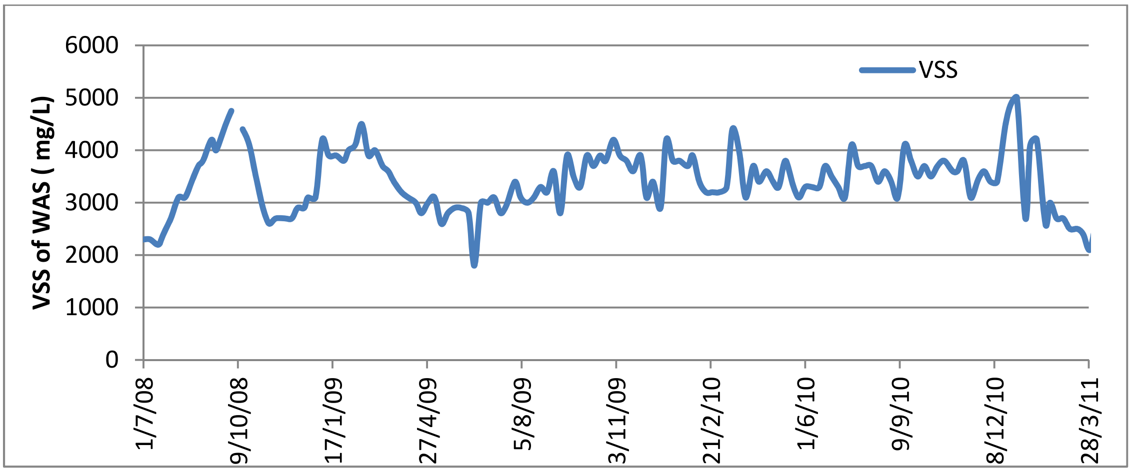


Figure 3-1: VSS of WAS in three-year period

As shown in Figure 3-2 the highest frequency of VSS occurs within the range of 2,875 to 3,125 mg/L and the second highest frequency range is 3,375 to 3,685 mg/L. Table 3-2 shows the average VSS of WAS is 3,376 mg/L. This information can be useful for the design loading of an anaerobic digester.

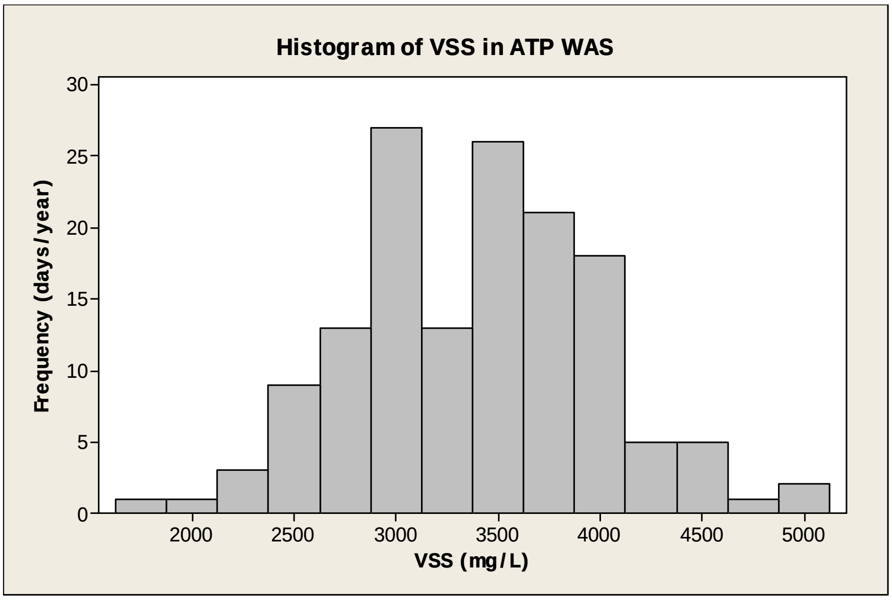


Figure 3-2: Frequency of ATP WAS VSS average concentrations

3.1.3. Aerobically Digested Sludge at ATP

As mentioned before, the WAS generated from IDEA at ATP is further treated using aerobic digestion. The characteristics of the aerobically digested sludge (e.g., pH, TSS, VSS and alkalinity) are given in Table 3-3.

Table 3-3: Characteristics of ATP Aerobically Digested Sludge

Parameters	pH	TSS	VSS	Alk-Total CaCO3	%VSS/TSS
Unit		mg/L	mg/L	mg $CaCO_3$/L	%
Average	7.043	7293	5957	305	81.8
Min	6.600	4700	3000	69	45.5
Max	8.3	9800	7800	660	86.3
SD	0.261	859.	710	73	4.1

3.1.4. Biosolids from ATP

Digested sludge is mixed with polymer, which is sent to the belt filter presses and is dewatered. The dewatered sludge is referred to as biosolids. The sampling point of dewatered biosolids is shown in Figure 2-2.

The chemical oxygen demand (COD) value for biosolids from July 2010 to April 2011 was made available to this study. Analysis of this data shows that the average COD of the dewatered biosolids is 1,056, 765 mg O_2/kg. Also the average VS of the biosolids are about 77.8% of the biosolids' total solids (TS).

Table 3-4: Characteristics of ATP Biosolids*

	COD (mg O_2/kg)	TS (w/w %)	VS (%TS)
Average	1056765	13.17	77.8
SD	508531.6	0.75	3.4
Max	3400000	15	83
Min	410000	12	72

*Analysis carried out at a NATA(National Association of Testing Authorities) accredited commercial lab through CWW

The ATP biosolids are usually characterised for heavy metals constituents to allow for their classification in accordance with the US Environmental Protection Agency (EPA) classes. The EPA regulation for biosolids application and treatment is discussed in section 3.2.3. The ATP biosolids concentration of copper and zinc were found to be higher than the upper limits of Grade C1 sludge, hence, the ATP biosolids are classified as Grade C2.

Table 3-5: ATP Biosolids Heavy Metals Concentration

		ATP biosolids		(EPA Victoria,2004)	
	(mg/kg)	Average	Max	Grade C1	Grade C2
Arsenic As		4	16	20	60
Cadmium Cd		1	1.9	1	10
Chromium Cr		12	66	400	3000
Copper Cu		**240**	**800**	**100**	2000
Mercury Hg		1	7	1	5
Nickel Ni		16	**78**	**60**	270
Lead Pb		15	48	300	500
Selenium Se		7	29	3	50
Zinc Zn		**387**	**1500**	**200**	2500
DDT		0	0.06	0.5	1
Organochlorine pesticides	Aldrin	<0.02	<0.05	0.05	0.5
	Chlordane	0.04	0.24		
	Dieldrin	<0.02	<0.05		
	Hexachloro-benzene	<0.02	<0.05		
	Heptachlor Epoxide	<0.2	<0.5		
Total PCB		<0.02	<0.05	0.2	1

3.2 Management of Sewage Sludge (Biosolids)

There are a number of pathways for the management of sludge generated at wastewater treatment plants. The sludge, after treatment and dewatering, is referred to as biosolids. There are regulations both on the national and state level for guidelines for the disposal of and beneficial use of biosolids, such as ‘Guidelines for Sewerage Systems Sludge (Biosolids) Management’ (NWQMS,2004), ‘Guidelines for Environmental Management: Biosolids Land Application’ (EPA Victoria,2004) and ‘Guidelines for environmental management: use of biosolids as geotechnical fill’ (EPA Victoria,2009).

Though the guidelines have many inconsistencies from state to state, they share the same basic structure in that they mandate contaminant standards, pathogen and vector attraction reduction standards, management practices and sampling and reporting requirements.

3.2.1. **Biosolids Classification**

Biosolids are classified into different grades according to the concentration of the constituent contaminant. Table 3-6 below shows the contaminant upper limit for biosolids classification selected contaminants according to the state, national and European regulations. The European standard (European Commission,2001) is included to compare the international contaminant upper limits with Australian guideline values. The biosolids from ATP are classified as Grade C2 based on its mental ion concentration. Detailed data can be found in section 3.1.4.

Table 3-6: Contaminant Upper Limits for Biosolids Classification

mg/kg$_{\text{dry weight}}$	**EPA Victoria (EPA Victoria,2004, 2009)**			**National Australian Guidelines(NWQMS,2004)**		**Europe limits 86/278/EEC (European Commission,2001)**
Contaminant	Grade C1	Grade C2	For geotechnical reuse	Grade C1	Grade C2	
Arsenic (As)	20	60	500	20	60	-
Cadmium (Cd)	1	10	100	1	10	20-40
Chromium (Cr)	400	3000	500	100-400	500-3000	-
Copper (Cu)	100	2000	5000	100-200	2500	1000-1750
Lead (Pb)	300	500	1500	150-300	420	750-1200
Mercury (Hg)	1	5	75	1	15	16-25
Nickel (Ni)	60	270	3000	60	270	300-400

Zinc (Zn)	200	2500	35,000	200-250	2500	2500-4000
Selenium (Se)	3	50	No data	3	50	
DDT & derivatives	0.5	1	No data	0.5	1	
Organochlorine pesticides	0.05	0.5	1	0.02-0.0.5	0.5	
PCBs	0.2	1	2	0.05-0.3	0.5	0.2-1

3.2.2. **Sludge Treatment Classification**

Treatments with varied technologies and conditions are also classified into grades based on their ability for reducing pathogen levels and regrowth potential. The specific sludge treatment classification with its process description and associated controls according to EPA Victoria is given in Appendix 2.

Aerobic digestion for more than 40 days is required for the process to be qualified as treatment Grade T3, whereas thermophilic AD could be considered treatment Grade T1, and mesophilic AD for 15 days is classified as treatment Grade T3.

3.2.3. **Biosolids Beneficial uses**

The beneficial uses of biosolids for land application depend on the quality/classification of the biosolids, as well as the grade of treatment applied (see Appendix 3). Given the ATP current biosolids data (details in section 3.1.4), the biosolids should be classified as grade C2, and its current eight-day aerobic sludge treatment is classified as grade T3 or worse. Hence, based on the Appendix 3 (adopted from EPA Victoria, 2004), current ATP biosolids should only be used either for: (1) processed food crops, (2) sheep grazing and fodder (also horses, goats), on food crops, woodlots or (3) landscaping, (restricted public access), forestry and land rehabilitation.

The control measures of contaminates minimisation in the biosolids are not in the scope of this project, especially the heavy metals. However, by recovering energy from sludge through AD, it may achieve low pathogen levels with minimum regrowth potential (Grade T1 or T2) due to its higher process temperature of AD. With the higher grade of the treatment, there will be more possibilities for beneficially using the treated sludge.

3.3 Technologies for Energy Recovery from Sludge

There are a number of physical, biological and chemical processes available for energy recovery from sludge. Those processes can be used to convert sludge into biogas, syngas or oil, which is then further converted into an energy source such as electricity, heat or biofuel, respectively. For example, technologies for recovering energy from sludge include AD, pyrolysis, gasification and incineration.

3.3.1. **AD**

AD is a well-developed biological process. It degrades organic matter into methane and carbon dioxide or 'biogas' by a mixed consortium of anaerobic microorganisms in the absence of oxygen.

In 1776, Volta was the first recognised anaerobic biological process that was a method to convert waste into energy (Barker et al.,1956). Further, Reiset studied the decomposition of organic matter in 1860 (Buswell et al.,1938). The first patented septic tank using anaerobic application was installed in 1895 in England with a capacity of 230 m^3/L (Cameron et al.,1899). A two-stage anaerobic process was first fully running in 1904 in Germany (Metcalf et al.,2003; Metcalf, L et al.,1915).

AD is generally favourable for sludge management at large WWTPs (i.e., of capacity above 20,000 to 30,000 persons equivalent [PE]), as energy recovered by exploiting the methane gas produced would be economically feasible (Foladori et al.,2010). The ATP is 52,000 PE, therefore, it is worth assessing the feasibility of biogas production from ATP sludge, which is the aim of this study.

3.3.2. **Pyrolysis**

Pyrolysis is the thermal decomposition of the sludge under high temperature and in the absence of oxygen. During the pyrolysis process, the organic fraction of the sludge being thermally decomposed is converted into char, pyrolysis oil and gas. The process occurs at a temperature of 600°C or less. The oil can be used as industrial fuel or to run a generator for electricity production. The char can be further gasified into syngas.

3.3.3. **Gasification**

Gasification is one of the processes to convert the sludge into an energy rich syngas. It utilises one of the pyrolysis products. The char generated in the pyrolysis step is further gasified in the presence of oxygen at temperatures above 850°C and produces a gas called syngas. Typical syngas contains nitrogen (N_2), carbon dioxide (CO_2) and carbon monoxide (CO) and hydrogen (H_2).

The principal of pyrolysis and gasification was first introduced in 1958 at Bell Laboratories in the US. Since then, numbers of different processes based on pyrolysis and gasification have emerged in last few decades. However, the technology has not yet been commercialised in Australia. A pilot gasification facility for green waste opened in Wollongong, New South Wales, in 2001, but subsequently closed down due to financial difficulties. However, full scale installations have been constructed in Germany (Kalogo et al.,2008).

3.3.4. **Incineration**

Incineration is another technique converting sludge to syngas, which is also called combustion or thermal oxidation. In this process the sludge is incinerated at a temperature of around 1,000°C in the presence of oxygen. Incineration is a commercially proven technology. The first incinerator was built in 1885 on Governor's Island, New York(USEPA,2002). The US EPA has reported there were 343 biosolids incinerators in the US in 1993 (Kalogo et al.,2008).

In Melbourne, Australia, AceWaste operates a high temperature incinerator treating hazard waste; the plant is in Dandenong, Victoria. Air pollution control and ash management are required to ensure the protection of the environment from the incineration's by-products. Overall, the sludge to syngas processes are relatively complex, use high temperature and are somewhat less sustainable than the AD process (Kalogo et al.,2008).

3.3.5. **Comparison**

There are different technologies available for the recovery of energy from waste. In order to determine the most feasible technology, a simple ranking method is shown in Table 3-7 and Table 3-8.

The ranking criteria include: (1) the development of the technologies, (2) operation temperature, (3) energy input and output, (4) equipment/system capital cost and (5) the operational and maintenance cost. The most developed technology is given a rank of 1, which is the highest score given. AD is the earliest developed technology among the technologies being investigated in this section. High temperature can cause difficulties in operation and high risk of incidents, thus technologies run at lower temperatures get a better score. As the aim of the use of these technologies is to recover energy, if the technology requires high energy input, most likely it will affect the net energy production. According to the data obtained from Kalogo (2008), AD scores the best as it requires the lowest energy inputs. Energy output measures the rate of energy recovery of the technology. The highest energy output is from pyrolysis. For technologies where data are not available, a low score is given. In terms of economic factors, AD has the lowest the initial capital cost and pyrolysis has the lowest operation and maintenance cost.

The total ranking is the summation of the scores for each option. As shown in Table 3-10, AD is the most feasible technology for energy recovering from wastes. Overall, AD is a well-developed technology, operates at a low temperature, uses relatively small amount of energy input and requires low capital cost for system installation. Therefore, the second phase of this study focused on AD.

Table 3-7: Characteristics of Technologies Available for Energy Recovery from Wastes

	Unit	Anaerobic Digestion	Pyrolysis	Gasification	Incineration
Technology development	First developed (year)	1776	1958	1958	1885
Temperature	°C	35 or 55 °C	600°C	850°C	1000°C
Energy input*	Kwh/dry MT	0.3-500	120-700	100	n.a.
Energy output*	Kwh/dry MT	6-1350	750-2000	1400	n.a.
Capital cost*	US$/dryMT	$530-1700	$1000-2000	n.a.	n.a.
Operation cost*	US$/dryMT.yr	$20-400	$80-120	n.a.	n.a.

*data obtained from (Kalogo et al.,2008)

Table 3-8: Ranking of Technologies Available for Energy Recovery from Waste

	Criteria for most feasible	Anaerobic digestion	Pyrolysis	Gasification	Incineration
Technology development	Well developed technology	1	3	3	2
Temperature	Low temperature for easy operation	1	2	3	4
Energy input	Low energy input for economical and economic benefits	1	3	2	4
Energy output	High energy input for economical and economic benefits	3	1	2	4
Capital cost	Low capital cost	1	2	3	3
Operation cost	Low operation cost	2	1	3	3
Total ranking		**9**	**12**	**16**	**20**

1 - the most feasible
4 - the least feasible

4. Stabilisation of Sludge by AD

Sludge stabilisation can be achieved by aerobic and AD. Compared with the current aerobic sludge treatment employed in ATP, the advantages and disadvantages of AD are as follows.

• Advantages of AD:

- Well-developed technology, applied since 1776
- Better sludge reduction compared with aerobic digestion
- Greenhouse gas emissions reduce from less waste sending to landfill
- Generates marketable products, such as energy and/or compost
- Minimal risk of health consequences as better pathogen reduction at a higher temperature compared with aerobic digestion
- Ad is a sustainable approach in terms of less energy input and energy gain
- If compared with sludge to syngas or to oil process, ad is operated under safe temperature and pressure.

•Disadvantages of AD:

- AD systems are more complex than aerobic digestion
- Long start-up time in order to obtain a given biomass concentration, compared with aerobic digestion process
- If the system is disturbed, it might take longer for the system to restore its normal working condition
- Restricted to treating organic materials, whereas combustion can destroy more solids
- Require trace metal ions to maintain optimum growth environment for methanogens, but not too much to cause toxicity
- Effluent might have higher COD and ammonia due to the nature of AD.

4.1 Principle of AD

The simplified AD reaction can be explained as:

$$\text{Organic matter} + H_2O \rightarrow CH_4 + CO_2 + \text{new cells} \qquad \textbf{Equation 4-1}$$

In the chemical Equation 4-1, organic matter reacts with water in the absence of oxygen/air and degrades into new cells, carbon dioxide and methane gas. The new cells generated are less than the original weight of organic matter since the partial of the carbon is converted into CO_2 and CH_4. By this means, the mass of the organic matter reduces. In the case of AD from sludge, the organic fraction of the sludge reduced. According to the US EPA, only between 0.04-0.1 g microbial mass in terms of VSS is formed per gram of BOD_5 removed (USA EPA,1979). The methane gas and carbon dioxide can be separated, then the pure methane is used for energy generation.

The mechanism of the AD of organics comprises three steps: (1) hydrolysis by extra cellular enzymes, (2) acidogenesis/fermentation by acid forming bacteria and (3) methanogenesis by methanogens. These steps are illustrated schematically in Figure 4-1.

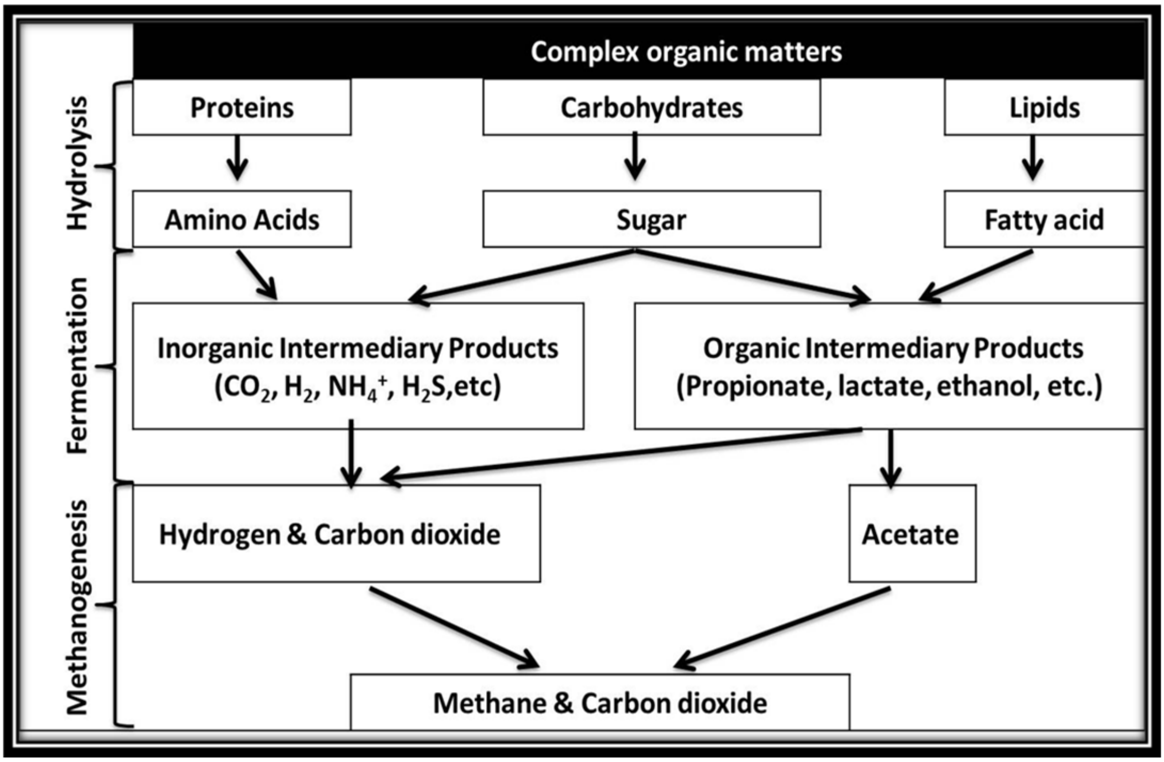


Figure 4-1: AD steps

- Hydrolysis

Hydrolysis is the first step where particulate materials become soluble and then can be further hydrolysed into simple monomers. This step has been found to be the limiting step in the speed of AD. Hydrolysis of sludge is slow as the step is based on endogenous metabolism (Foladori et al.,2010). Thus, increasing hydrolysis means more solubilised substrate readily being acidified and transformed into methane, which suggests a more efficient and fast digestion.

Chemical, mechanical and thermal pre-treatments of anaerobic feed have potential to shorten the digestion period through enhancing the hydrolysis step (Battimelli et al.,2010; Bougrier et al.,2008; Lei et al.,2007).

- Acidogenesis/Fermentation

The second step is acidogenesis/fermentation, where the soluble products from the first step are converted to a mixture of intermediary products such as acetate, hydrogen, CO_2, propionate and butyrate. The acetate and propionate are fermented further, which results in the production of acetate, hydrogen and CO_2 (Metcalf et al.,2003).

- Methanogenesis

In the third step, methanogenesis, acetate is biodegraded into methane and CO_2 by aceticlatic methanogens. Also, hydrogen-utilising methanogens produces methane from hydrogen and CO_2.

4.2 AD Types

4.2.1. Mesophilic Versus Thermophilic AD

The anaerobic process strongly depends on the temperature. Anaerobe showed highest efficiency at temperatures of 35 to 40°C for the mesophilic condition and at about 55°C for thermophilic conditions. Accordingly, the corresponding methanogens of those two conditions are classified as mesophilic and thermophilic (see Figure 4-2).

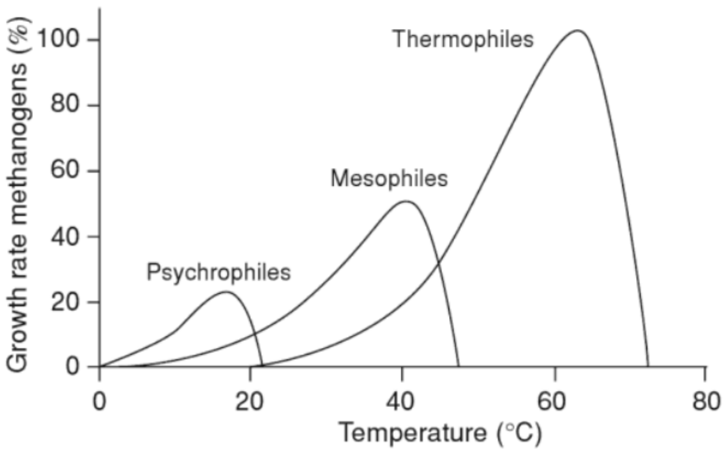


Figure 4-2: Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens (Lettinga et al.,2001)

In conventional mesophilic AD, about 40 to 50% of organic matter is degraded, usually leading to TSS reduction of around 30%, whereas conventional thermophilic AD can achieve similar VSS and TSS reduction within a shorter retention time. The higher VSS and TSS achieved using thermophilic AD, though, has to be weighed against greater energy consumption (Foladori et al.,2010). In other words, if digestion occurs under similar conditions such as the organic loading rate (OLR) and sludge retention time (SRT), thermophilic reactors would produce more biogas and remove more solids than mesophilic reactors (Coelho et al.,2011).

Thermophilic AD has a higher temperature range than mesophilic AD, hence, it achieves better reduction of pathogens compared to mesophilic AD (Mata-Alvarez et al.,2000). However, digestate from thermophilic AD has poor dewaterability characteristics (Coelho et al.,2011; Song et al.,2004) In addition, thermophilic methanogen are more susceptible to TAN inhibition than the mesophilic cultures (Poggi-Varaldo et al.,1997). Ammonia as a factor of AD reactors performance is discussed in section 4.3.2. Another disadvantage of thermophilic AD is that the effluent quality is poorer than the effluent from mesophilic AD (Song et al.,2004).

4.2.2. Temperature phased AD

Temperature phased systems provide the choice for employing both mesophilic and thermophilic AD conditions for two reactors in one system. The first phase/period of digestion is under thermophilic condition and the second phase/period is under mesophilic condition.

The two-stage phase-separated process combines the advantage of thermophilic digestion (55°C), which has better pathogen control and volatile solids (VS) reduction. The most economical operation conditions has been found when the two-stage process is optimised so that the bulk of the digestion takes place in the mesophilic stage (35°C) (Coelho et al.,2011; Han et al.,1997; Song et al.,2004).

4.2.3. Two-phase AD

Conventional AD uses a single-stage system, where both the acidogenesis and methanogenesis steps occur in the same reactor under either mesophilic or thermophilic condition.

Two-phase AD splits the acidogenesis and methanogenesis into two reactors operating in a series. The first reactor is designed to promote hydrolysis and fermentation, hence, is operated at a pH range of 5.5 to 6.0. The second reactor is designed to promote methanogenesis at a pH higher than 6 (Wang et al.,2008). By separating the AD process steps, each reactor can achieve the most effective breakdown of organic matter into methane gas, a shorter detention time and better biogas quality. The schematic diagram of two-phase AD is shown in Figure 4-3.

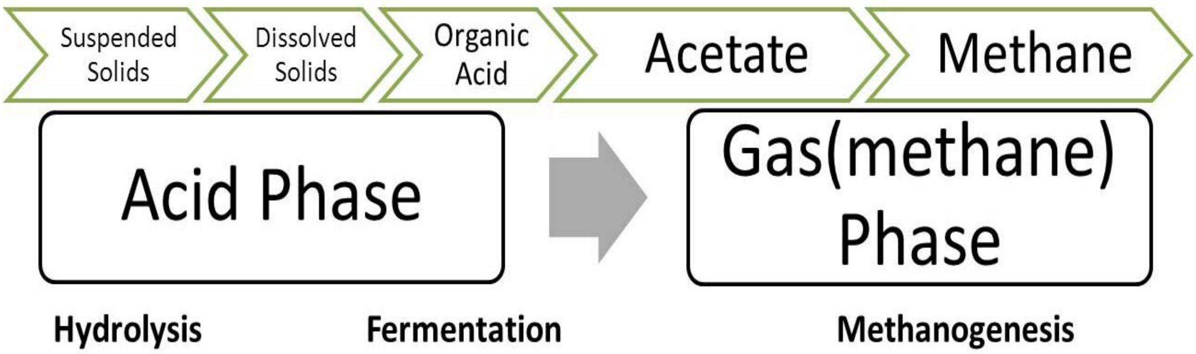


Figure 4-3: Schematic diagram of two-phase AD

4.3 Factors influencing AD

There are some factors that have been reported to inhibit anaerobic digestion, hence, have a negative effect on the AD reactor's performance, such as pH, ammonia concentration, volatile fatty acids and metal ions. The optimal combination of those parameters can provide the right environment for the AD microbial community, which then results in organics degradation, sludge stabilisation and high methane yield.

4.3.1. **pH and Alkalinity**

The anaerobes can be classified according to the pH range at which they function. These are the acidogens and methanogens, which perform best at pHs of 5.5 to 6.5 and 7.8 to 8.2, respectively. The combined anaerobic groups have an optimum pH of 6.8 to 7.4, with neutral being the ideal environment (Khanal,2009). Slight pH change can adversely affect the performance of the AD process. Acidogens are significantly less sensitive to low pH values; however, it is more important that methanogens are maintained close to neutral. If low pH (<7) occurs, it reduces the activity of methanogens, which can lead to the accumulation of acetic acid and H2 in the reactor. Once the acid fermentation step prevails over methanogenesis, the reactor contents become 'sour' and the pH drops further; the main products that have been under these conditions are acetic and butyric acid (Boe,2006). This could eventually cause the failure of the process.

The alkalinity in an anaerobic digester can be generated from the degradation of nitrogenous organic compounds, reduction of sulphate, increase of volatile fatty acids (VFA) and release of orthophosphate. However, the alkalinity level in the digester is mainly influenced by the influent sewage characteristics (Song et al.,2004). The average alkalinity of WAS from ATP is 265±37.5 $mgCaCO_3/L$ (data collected from June 2008 to March 2011).

4.3.2. **Ammonia**

Ammonia is the by-product from biodegradation of nitrogenous matter, mostly in the form of proteins and urea (Chen et al.,2008). Free ammonia has been reported to have an inhibitory effect on AD. Different mechanisms have been proposed to explain ammonia inhibition, such as the inhibition of specific enzyme reactions changing the intracellular pH due to the membrane-permeability for free ammonia. Factors controlling ammonia inhibition are ammonia concentration, pH, temperature, concentration of metal ions and acclimation of the inoculum.

Ammonia concentration below 200 mg/L is generally considered advantageous for AD as nitrogen is an essential nutrient for the microorganisms. Some sensitive methanogenic strains such as *Methanospirillumhungatei, Methanosarcinabarkeri,and Methanobacteriumhermoautotrophicum* are reported to be inhibited at 4.2gN/L(Jarrell et al.,1987).

Acclimation can happen during AD when the population of methanogens changes. If less sensitive strains become the predominant species, ammonia inhibition could be reversed. It is reported that methane was produced at 11 g N/L after adaptation, whereas the acclimated methanogens failed at 1.9 to 2 g N/L (Koster et al.,1984).

Free ammonia of 560 to 568 mg N/L is reported to cause a 50% inhibition of Methanogenesis at pH 7.6 under thermophilic conditions (Sung et al.,2003). Ammonia nitrogen inhibition concentration of 1400-1700 mg/L is reported at AD of kitchen waste (Pan et al.,2011).

Higher pH can increase the ratio of free ammonia to ionized ammonia (NH^{4+}), and free ammonia is toxic to microorganisms. Acidification of waste to lower pH could maintain free ammonia at a safe level, and also reutilise VFAs.

Free ammonia concentration can also be affected by temperature. Thermophilic digestion at 50 degrees is reported to be more easily inhibited compared with mesophilic digestion at 35 degrees due to increased free anomia concentration (Koster et al.,1984).

Presence of some metal ions such as Na+, Ca+ and Mg+ is reported as being antagonistic to ammonia inhibition. Phosphoriteore was found to stimulate biogas production when NH_4Cl is as high as 30 g/L; however, recovery from inhibition cannot be restored at the concentration of 50 g/ L (Krylova et al.,1997).

On the other hand, ammonia inhibition on AD reactors treating meat industry waste was also reported. For instance, Niu (2013) suggested biogas production reduced by 20% when TAN was increased to 10,000 mg N/L and the AD process was totally suppressed at TAN of 16,000 mg N/L. Krylova (1997) also reported 80-90% of methane was suppressed at TAN of 8000 mg/L.

4.3.3. **VFA and LCFA**

Fatty acids are the intermediates formed in the AD process as a results of the degradation of lipids and fats during the hydrolysis step. Fatty acids are then further degraded into acetate and hydrogen. VFA are the fatty acids with a carbon chain of six carbons or fewer, whereas long chain fatty acids (LCFA) are the fatty acids with aliphatic tails longer than 12 carbons. Both VFA and LCFA have been found to inhibit AD under certain concentrations. VFA can be toxic to microorganisms especially to methanogens at a concentration of 6.7 to 9.0 mol/m^3 (equivalent to 400 to 550 g/L for C2 and C3) (Batstone et al.,2000), and LCFA was reported to cause permanent inhibition at much lower concentrations, such as 1.0 g/L (Angelidaki et al.,1992).

The toxicity of VFA is due to its undissipated form that is freely membrane-permeable, so that a disruption of homoeostasis is made. Also the ambulating the VFA results in a pH reduction, and as mentioned in section There are some factors that have been reported to inhibit anaerobic digestion, hence, have a negative effect on the AD reactor's performance, such as pH, ammonia concentration, volatile fatty acids and metal ions. The optimal combination of those parameters can provide the right environment for the AD microbial community, which then results in organics degradation, sludge stabilisation and high methane yield.

pH and Alkalinity the low pH condition is not favourable for methanogens. The mechanism of the LCFA toxicity is caused by adsorption onto the cell wall or cell membrane of anaerobes, which interferes with the transport and/or protection functions of the microorganism (Appels et al.,2008).

4.3.4. **Cationic Ions**

Cationic elements, such as Na+, K+, Mg2+, Ca2+ and Al3+, are brought into the digester from the feed flow and the compounds are added for pH adjustment. These ions increase the salt level, which will cause bacterial cells to dehydrate due to osmotic pressure. Moderate concentration of salt can provide a favourable environment for microbial growth; however, the growth slows down by excessive amounts and extremely high levels of some metals can result in high toxicity. The potential inhibition level of metal ions to AD according to EPA US is shown in Table 4-1:

Table 4-1: Substances with Potential to Cause Biological Inhibition in AD (EPA US,2006)

Substance	Moderately Inhibitive (mg/L)	Strongly Inhibitive (mg/L)
Calcium (Ca)	1,500–4,500	8,000
Magnesium (Mg)	1,000–1,500	3,000
Sodium (Na)	3,500–5,500	8,000
Potassium (K)	2,500–4,500	12,000
Ammonia Nitrogen	1,500–3,000	3,000
Copper (Cu)	—	50–70 (total)
Chromium VI	—	200–250 (total)
Chromium	—	180–420 (total)
Nickel (Ni)	—	30 (total)
Zinc (Zn)	—	1.0 (soluble)

According to the literature, 200 mg/L of Ca^{2+} is the optimum concentration for methanogenesis of acetic acid (Kugelman et al.,1964), 350mg/L of Na^{+} is the optimal concentration for growth of hydrogenotrophic methanogens, and K^{+} below 400 mg/L improves the function of anaerobes (Appels et al.,2008).

4.3.5. Retention Time

Hydraulic retention time (HRT) is the time that the hydraulic flow spends in the reactor; whereas, the solids retention time (SRT) is the average time solids spend in the digester. For a completely stirred tank reactor where liquid and solids/biomass are mixed together in the outflow, the SRT and HRT are the same.

A schematic representation of biogas yield production versus SRT is shown in Figure 4-4 . Retention times shorter than five days are insufficient as the methanogenic bacteria are wasted in outflow and cause accumulation of VFA. In order to break down the compounds, longer retention time (SRT>10 days) is needed especially for compounds of low biodegradability such as lipids and fat. SRT is the fundamental design and operating parameter for AD.

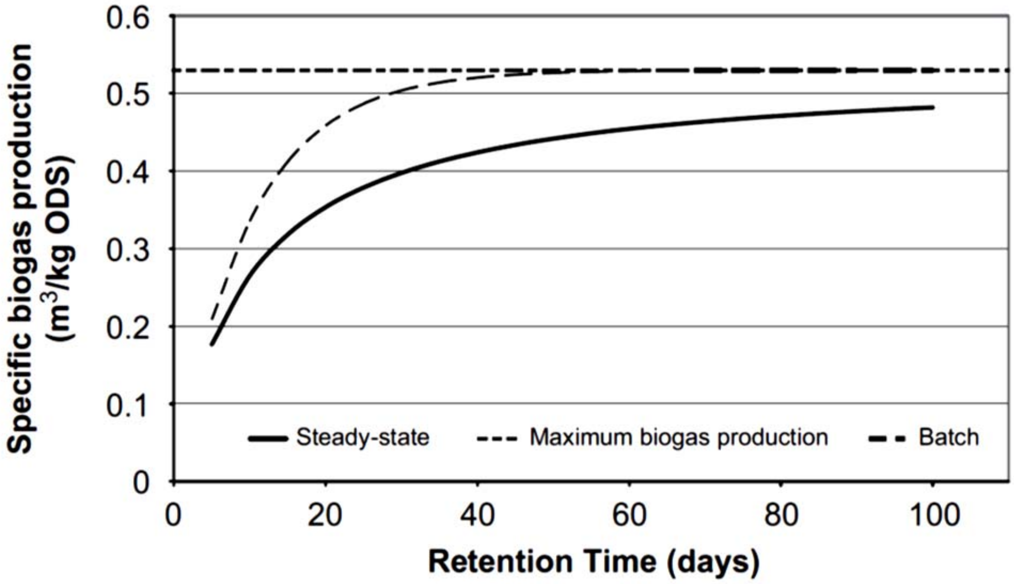


Figure 4-4: Biogas production versus SRT (Appels et al.,2008)

4.4 Substrates for AD

AD has not only been studied for stabilisation of sludge generated from wastewater treatment (i.e., WAS), but is also used for treatment of organic waste materials, such as grease trap sludge, meat industry or slaughter house waste and organic fractions from municipal solid waste. There is wide range of substrates suitable for AD treatment.

4.4.1. **WAS**

AD of WAS is well studied as indicated by the number of publications in this area. Table 4-2 lists the summary of the findings reported in some publications. However, there are limited numbers of papers that looked into stabilisation of sludge from IDEA activated sludge systems. Conversely, the IDEA system is becoming popular. As of 2003, there were seven sewage treatment plants utilising IDEA activated sludge systems in Victoria (GHD,2003). More systems have been installed since then, in the process of sewage treatment plants upgrading to achieve target nitrogen removal. For instance, the ATP commissioned its IDEA tank to replace a trickling filter in 2006 (VicWater,2007), and the Portland wastewater treatment plant will have an installation of an IDEA system completed by mid-2013 (VicWater). The IDEA process generates slightly more stabilised WAS compared to WAS from conventional activated sludge processes, as the aeration period is longer. Therefore, this current project investigated the biogas potential from IDEA WAS.

Table 4-2: AD of WAS in the Literature

Reference	Waste type	OLR	Condition	Reactor size	HRT (day)	Biogas yield (mL/gVS_{added})	Solids removal
(Bolzonella, David et al.,2005)	IDEA WAS	1 gVS/L.d	Continuous mesophilic	Full scale WWTPs	22	180	
(Bolzonella, D. et al.,2007)	sludge of 3% TS		Batch mesophilic	1 L		240	
			Batch Thermophilic			430	
			Batch TPAD		2 (1st phase)	370	
(Carrere et al.,2012)	sewage sludge(3.9% TS)	1.8 gCOD/L.d	Semi-Continuous mesophilic	2L	21	116	33%
(Davidsson et al.,2008)	sludge of 4.1% TS	2.5gVS/L.d	Semi-Continuous mesophilic	Pilot-scale 35 L	13	271mLCH_4/gVS	45% VS
(Ge et al.,2011a)	sludge of 2.5% TS	1.1 gVS/L.d	Semi-Continuous TPAD	0.4L (1st phase) +4.2L (2nd phase)	2d(1st phase) +14d(2nd phase)	450	30-40%
(Kalloum et al.,2011)	sludge from lagoon	16 gTS/L	Batch mesophilic	1L	33	17.52 NmL/g TS	81% TS
(Luostarinen et al.,2008)	sludge of 3-5% TS	1.56-2.09 gVS/L.d	Semi-Continuous mesophilic	5L	16	441	52% VS
(Noutsopoulos et al.,2013)	(Noutsopoulos et al.,2013)sludge of 5.4% TS	1.9 gVS/L.d	Semi-Continuous mesophilic	3L	15	452	52 %VS
(Silvestre et al.,2011)	sludge of 3.2% TS	1.5 gVS/L.d	Semi-Continuous mesophilic	7L	20	346	36%VS
(Song et al.,2004)	sewage sludge	0.86 gVS/L/d	Semi-Continuous TPAD	5L + 13.6L	5d+6.7d	468mL/g VS removed	
(Wan et al.,2011)	sludge of 5.9% TS	2.34 gVS/L.d	Semi-Continuous mesophilic	4L	15	387	40% vS

4.4.2. **Meat Industry Waste**

Meat industry produces wastewater that usually contains high organic fractions. The type of waste depends on the process, such as animal washing, bleeding out, skinning and facility cleaning. In terms of content, the waste may contain blood, manure, poultry feathers and other animal by-products, which are high in organic content and rich in lipids and protein.

Researchers have shown that meat industry waste is a good resource for energy recovery though the AD process. A summary of the findings reported in the research is listed in Table 4-3.

It is noteworthy that AD of meat industry waste generally has longer HRT compared with AD of WAS. For example, the HRT of AD reactors treating WAS ranged from 13 to 22 days (see Table 4-2), whereas the HRT of AD reactors treating wastes from the meat industry have HRT varying from 21 to 50 days (see Table 4-3). This is due to the complicity of the organic matter present in the meat industry waste, such as fat and lipids, which are much less found in the WAS.

Further, AD of the meat industry waste, being rich in organics, lipids and proteins, usually suffers from inhibitions due to the accumulation of ammonia and acetic acid, released during the hydrolysis of these constituents. Salminen (2002b) reported that when the organic loading of the AD reactor increased from 0.8 to 1.0 kg VS/m$^{3}\cdot$d, the performance of the reactor deteriorated, indicating inhibition.

Codigestion of meat industry waste with other substrates is suitable, so that its high organic loading will be diluted to make AD possible.

Table 4-3: AD of Meat Industry Waste in the Literature

Reference	Waste type	OLR	Condition	Reactor size	Retention Time	Biogas Production	Methane	Comment
(Cuetos et al.,2008)	Slaughterhouse waste +OFMSW(1:5)	1.85 kg VSS feed/m3/d	Semi-continue Mesophilic	3L	25 days	8.6L/day	500 mL/gVS	
(Hejnfelt et al.,2009)	5%pork by products + 95% pork pig manure	8.3 gVS/d	Semi-continue TPAD	3.2L	21 days	660 L/kg	489 mL/gVS	Co-digestion is suitable for high organic loading waste up to a dilution level of 5%.
(MJ. Cuetos et al.,2009)	poultry blood + OFMSW (1:2V/V)	1.5 kg VSS feed/m^3.d	Semi-continue Mesophilic	-	36 days	0.33 m^3/kg VSS feed	200 mL/gVS	
(Salminen, EA et al.,2002b)	Poultry Slaughterhouse waste	0.8 kg VSS feed/m^3·d	Semi-continue Mesophilic	2L	50 days	-	520 mL/gVS	Increase loading to 1-2 kg VS/m^3.d and HRT of 25 to 13 days was found inhibited
(Woon et al.,2012a)	DAF sludge	3.38 gCOD/gVS	Batch Mesophilic	250mL	60 days	0.53 m^3/kg VSS feed	339 mL/gVS	
(Woon et al.,2012b)	DAF sludge	4.2-1.67 kg VS/m^3·d	Semi-continue TPAD	480mL	28 days	0.121 m^3/kg VSS feed		
		2.54 kg VS/m^3·d	Semi-continue Mesophilic	480mL	30 days	0.026 m^3/kg VSS feed		

4.4.3. **Codigestion**

Conventionally, AD is a single substrate treatment. Studies have found that AD of a variety of substrates is more stable than a conventional system and can achieve better biogas yield (Alvarez et al.,2008; Buendia et al.,2009; Davidsson et al.,2008; Luste et al.,2010). Codigestion of WAS with other wastes could enhance the biodegradability (Buendia et al.,2009) as well as increase the methane yield (Davidsson et al.,2008).

In order to enhance the energy production, codigestion of WAS from ATP with waste that has a high carbon content can increase the biogas production and improve the methane yield. Waste from the meat industry is abundantly available in the City West Service Area, which contains plentiful carbon sources, hence, high methane generating potential. Though this waste has potential for energy production, it is disposed at a waste handling facility and most often the industry has to pay for the waste disposal cost.

Codigestion of WAS and meat industry waste can create a win-win situation so that the meat industry could save costs on waste disposal and ATP could increase its biogas yield as well.

Table 4-4: AD of WAS Codigestion of Other Waste in the Literature

Reference	(Davidsson et al.,2008)	(Zhu et al.,2011)	(Wan et al.,2011)	(Carrere et al.,2012)
Waste type	Sewage sludge (4.1% TS)	Sewage sludge (1.9gVS/L)	Sewage sludge (5.9%TS)	Sewage sludge(3.9% TS)
	grease trap sludge (GTS) (17.3%TS)	GTS (5.5gVS/L)	fat, oil and grease(FOG) (3.2%TS)	fatty wastewater (4.3% TS)
OLR	1.7 gVS/Lday	7.4gVS/L	2.34gVS/L	1.8 gCOD/L.d
Condition	Semi-continuous mesophilic	Batch mesophilic	Semi-Continuous mesophilic	Semi-Continuous mesophilic
Reactor size	2L	Effective 100mL(125mL glass vessels)	4L	2L
Retention Time	10 days	27 days	15 days	21 days
Biogas Methane	(69% CH4) 344 mL/gVS	880 mL/gVS	(66.8% CH4) 598 mL/gVS	(71% CH4) 362 mL/gVS
Comment	27% methane increase when adding 30% of GS (VS based)	65% increased methane production with 4% (v/v) GTS addition	137% higher methane yield when codigestion with FOD (64% of VS)	212% increased methane production with 40% fatty wastewater added.

5. Materials and Methods

This section describes the materials, methods and experimental procedures used in the research.

5.1. Materials

The wastes being tested in this research included WAS and biosolids from ATP and DAF sludge from a rendering plant in Melbourne. All waste were characterised after being received from industries and before the experimental works being carried out. Variation in characteristic was observed over the experimental periods, especially the DAF sludge from the meat industry. For instance, the VS content of DAF sludge varied from 151 gVS/L to 48gVS/L during 4 months of the semi-continuous mesophilic AD and Temperature Phased Anaerobic Digestion (TPAD) of raw DAF sludge test. The characteristics of the samples fed into anaerobic digesters were used for calculation on AD performance and normalized biogas yield.

5.1.1. **WAS**

The WAS sample used in experiments was collected from the sludge outlet from TA102 at ATP. Characteristics of the WAS sample are shown in Table 5-1.

In the BMP from WAS (batch test), WAS settled for 20 mins, and the testing sample was taken from the bottom thickened layer. Later the results showed the reactor was under loaded (see section 6.1.1). Thus, in the mesophilic AD and TPAD codigestion from WAS (semi-continuous condition), the settling time of the WAS sample extended into 24 hours and the bottom thickened layer was taken for testing.

In mesophilic AD and TPAD codigestion from WAS and DAF sludge (semi-continuous condition), WAS functioned as a solvent to DAF sludge of low TS, therefore, the testing WAS sample was not thickened in this case.

Table 5-1: Characteristics of the WAS sample used in experiments

	Sample collection date										
	15/8/11	9/10/11	7/11/11	31/1/12	11/3/12	25/3/12	1/4/12	18/5/12	26/5/12	7/6/12	5/7/12
Parameters (Unit)	BMP from WAS (Batch test) *	Mesophilic AD & TPAD codigestion of WAS and DAF sludge (Semi-continuous condition)			Mesophilic AD & TPAD Codigestion of WAS (Semi-continuous condition)#						
TS (mg/L)	15,714	7,950	9,500	7,950	14,902	14,900	23,696	18,900	20,381	20,500	21,100
VS (mg/L)	10,619	4,628	4,800	3250	9,020	9,300	16,087	11,600	14,190	14,300	14,500
pH	6.68	6.50			6.05						
CODt (mg O_2/L)	13,920	6,360	4,100	5,760	19,280	41,400			22,820		
CODs (mg O2/L)	280	170			280						
TP (mg P/L)	749	271			1106						
TN (mg N/L)	1640	340			1450						
TAN (mg N/L)	1.4	4.1			2.7						
TVFAs (mg AceticAcid/L)	10										

* WAS sample was allowed to settle for 20 min to thicken it. # WAS sample was thicken by gravity for 24 hours, and the bottom layer was taken out for test

5.1.2. **Biosolids**

The ATP biosolids samples were collected after the BFP (belt filter press). Literature reported that conventional complete mix AD require TS content lower than 10%. (Battistoni et al.,2001a; Battistoni et al.,2001b; Battistoni et al.,1998; Fantozzi et al.,2011; Pavan et al.,2000; Pavan et al.,1994; Pavan et al.,1998). Hence the Biosolids sample was diluted before fed into reactors.

In batch test of Biosolids, the every 250 gram (0.25 kg) of biosolids was diluted with tap water into 1 Litre of biosolids slurry. As the BMP from WAS (Batch test) showed reactors were under loaded, so that the loading of the batch test from Biosolids were designed to be higher. In Semi-continuous Mesophilic AD and TPAD from biosolids tests, in order to compare biogas potential from the different substrates (i.e., thickened WAS and diluted DAF sludge, Biosolids sample was diluted to such that the VS for feeding slurry were similar with other materials used in the AD. Thus, the VS concentration selected was consistent with the VS concentration of thickened WAS applied for mesophilic AD and TPAD of WAS experiment (i.e., every 150 gram (0.15 kg) of biosolids diluted into 1 litre Biosolids slurry with tap water. The characteristics of the wastes before and after dilution are given in Table5-2 below.

Table 5-2: Characteristics of Biosolids used in experiments

Experiment	**BMP from Biosolids(Batch test) BMP from DAF sludge and Biosolids (Batch test)**		**Semi-continuous Mesophilic AD and TPAD of Biosolids**	
Sample date	9/9/11		28/9/12	
	Biosolids	Biosolids slurry*	Biosolids	Biosolids slurry#
TS	12.7% Dry solids	26,158 mg/L	14.4% Dry solids	21,571 mg/L
VS	82.6% TS	21,618 mg/L	81.5% TS	17,570 mg/L
pH	-	6.94(250g/L)	-	7.55
CODt	1,197,331 mg O_2/kg	31,320 mg O_2/L	1,094,361 mg O_2/kg	27,893 mg O_2/L
CODs	61,166 mg O_2/kg		55,974 mg O_2/kg	1,426 mg O_2/L
TAN	2,752 mg N/kg	72.0 mg N/L	2,542 mg N/kg	64.8 mg N/L
TN	110,099 mg N/kg	2,880 mg N/L	60,682 mg N/kg	1,160 mg N/L
TVFAs			8,370 mgAceticAcid/kg	160 mgAceticAcid/L

* 250g of Biosolids making up 1L of Biosolids slurry with tap water; #150g of Biosolids making up 1L of Biosolids slurry with tap water

5.1.3. **DAF Sludge**

DAF sludge sample was collected from a rendering plant in west Melbourne. The plant produced about 1.1ML/day of wastewater, of, which 50% is contaminated process condensate as the major contributor to wastewater load is the discharge of condensates and stick water/raw material losses to sewer. Overall, the wastewater production (average of 2005-2006) on the site is 321ML/yr, of, which contains 1038 tons BOD, at average concentration of 3250 mg/L, 624 tons of dissolved solids (TDS) at average concentration of 1950 mg/, 46 tons of suspended solids, at average concentration of 140 mg/L and 202 tons of nitrogen, at average concentration of 630 mg/L.

In terms of the DAF sludge, the production rate is about 2700 tons annually and on average 10 tons per day for 5-working days each week. The schematic diagram of DAF sludge production is shown in Figure 5-1.

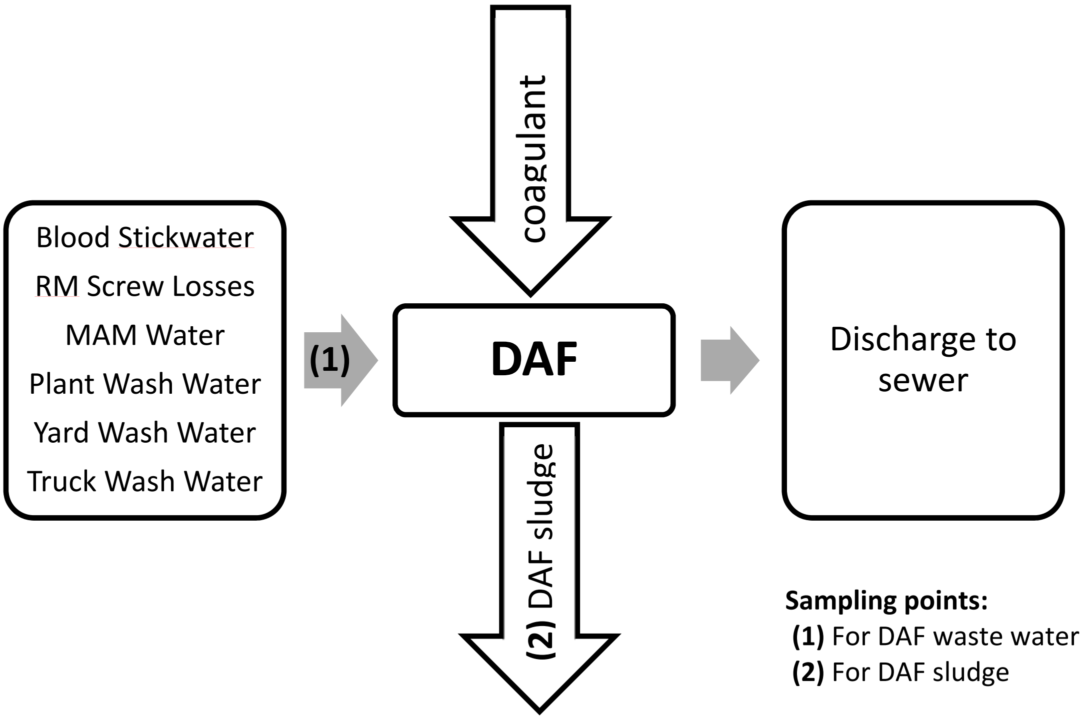


Figure 5-1: Schematic diagram of DAF sludge

5.1.3.1. ***DAF sludge***

The DAF sludge samples were collected from the DAF sludge tankage in the meat industry and being characterised before testing, the result can be found in Table 5-3.

Table 5-3: Characteristics of the DAF sludge sample used in experiments

Parameters (Unit)	Sample collection date												
	9/9/2011	9/10/11	8/11/11	7/12/11	11/1/12	11/3/12	25/3/12	1/4/12	5/13/12	26/5/12	7/6/12	5/7/12	28/9/12
	BMP from DAF sludge and Biosolids (Batch test)	Mesophilic AD & TPAD Codigestion of WAS and DAF sludge (Semi-continuous condition) Mesophilic AD of DAF sludge of low TS (Semi-continuous condition with raw inoculum)				Mesophilic AD & TPAD of raw DAF sludge (Semi-continuous condition) TPAD of DAF sludge of low TS (Semi-continuous condition)							Mesophilic AD of DAF sludge of low TS With Acclimated Inoculum
TS (mg/L)	47,300	142,950	121,200	77,968	113,818	151,238	142,100	121,850	115,300	101,714	102,000	57,000	66,049
VS (mg/L)	40,500	136,024	113,900	73,272	105,182	139,810	130,600	110,700	104,200	92,095	94,900	48,100	58,868
VS/TS	86%	95%	94%	94%	92%	92%	92%	91%	90%	91%	93%	84%	89%
pH	5.65	5.10				5.68							6.16
CODt (mg O_2/L)	141,600	306,400	291,000	187,200	257,750	237,400				204,200			157,800
CODs (mg O_2/L)	16,570	8,590				32,540							9,040
TP (mg P/L)	4930	3456				1496							1236
TN (mg N/L)	4560	3200				1760							2080
TAN (mg N/L)	1328	864				1304							452
TVFAs (mg AceticAcid/L)	3075	2966				-							3,292

*

5.1.3.2. *Non-polymer DAF sludge*

In production of the DAF sludge, polymer is added to thicken the sludge for easy collection. Polymer has been reported to cause significant reduction in anaerobic biodegradability with polymers at acetylation levels of between 1.2-1.7 (Rivard et al.,1992).

In order to study the effect of the polymer on AD process, a synthetic DAF sludge without polymer in the lab, so called non-polymer DAF sludge was prepared to compare with the DAF sludge sample from the industry. The non-polymer DAF sludge here refers to the concentrated of the DAF wastewater by centrifuge without polymer.

Also, pervious tests showed the DAF sludge of low TS and long HRT reactors yielded higher amount of biogas as well as in better organic reduction. Hence the non-polymer DAF sludge was diluted to similar organic content of the thickened WAS diluted Biosolids and diluted DAF sludge. Characteristics of the non-polymer DAF sludge before and after dilutions are shown in Table 5-4 and Table 5-5.

Table 5-4: Characteristics of the Non-polymer DAF sludge prepared in the lab on 11/3/12

Parameters (Unit)		Before dilution 11/3/12	After dilution 11/3/12
TS	mg/L	47,500	13,524
VS	mg/L	44,100	12,286
pH	pH	5.87	5.93
CODt	mg O_2/L	120,600	38,940
CODs	mg O_2/L	5,960	1,220
TP	mg P/L	896	894
TN	mg N/L	480	1080
TAN	mg N/L	332	72

Table 5-5: Characteristics of the Non-polymer DAF sludge (before and after mixng with water to lower the TS) tested in the experiment of Mesophilic AD and TPAD of Non-polymer DAF sludge

	Parameters (Unit)	Sample collection date 11/3/12	25/3/12	1/4/12	25/4/12	13/5/12	26/5/12	7/6/12	5/7/12
Before mixing	**TS** (mg/L)	47,500	84,800	57,573	124,100	106,700	63,810	87,500	74,000
	VS (mg/L)	44,100	80,200	54,369	119,200	101,458	61,905	79,500	67,500
After mixing	**TS** (mg/L)	13,524	10,600	14,571	16,066	13,900	12,762	17,500	14,800
	VS (mg/L)	12,286	10,025	13,761	15,432	12,500	12,381	15,900	13,500

5.1.4. **Anaerobic Inoculum**

Anaerobic inoculum is referred either as inoculum or seed in some literatures. It brings in the anaerobic microorganisms into the reactor and speeds up the start-up of the AD process.

5.1.4.1. ***Raw Inoculum***

Anaerobic raw inoculum was obtained from anaerobic digesters of Wastewater treatment Plants in Melbourne, Australia. The anaerobic inoculum was also sieved through a screen of 1.0mm mesh size and stored at 35◦C in the oven for three days before its characterisation and the AD experiments being carried out.

Characteristics of the raw inoculum used in each experiment are shown in Table 5-6.

Table 5-6: Characteristics of the anaerobic raw inoculum used in this study

		Testing date				
		15/8/11*	9/9/2011#	9/10/11*	11/3/12*	28/9/12#
Test conditions		Batch tests		Semi-continuous conditions		
Experiment		BMP from WAS;	BMP from Biosolids; BMP from DAF sludge and Biosolids;	Mesophilic AD of DAF sludge of low TS; Mesophilic AD &TPAD codigestion of WAS and DAF sludge;	Mesophilic AD & TPAD of WAS ; Mesophilic AD & TPAD of raw DAF sludge ; Mesophilic AD of non-polymer DAF sludge; TPAD from DAF sludge of low TS;	Mesophilic AD & TPAD of biosolids; Comparison for mesophilic AD of DAF sludge of low TS with acclimated inoculum;
Parameters	(Unit)					
TS	mg/L	19,619	35,700	18,350	24,608	25,670
VS	mg/L	13,429	26,500	13,324	14,608	18,350
pH	pH	7.31	8.16	7.15	7.3	8.17
CODt	mg O_2/L	23,430	50,925	21,860	27,340	28,180
CODs	mg O_2/L	1,840	1,400	414	500	640
TP	mg P/L	950	2,328	1,916	1,744	1448
TN	mg N/L	2,515	3,000	1,740	1,280	2,160
TAN	mgN/L	700	822	396	552	320
TVFAs	(mg AceticAcid/L)	69	-	11	-	64

*Anaerobic raw inoculum was obtained from Eastern Wastewater Treatment Plant (ETP) in Melbourne, Australia
#Anaerobic raw inoculum was obtained from Melton Recycled Water Treatment Plant (Melton RWP) in Melbourne, Australia

5.1.4.2. *Acclimated DAF Inoculum*

AD with acclimatised inoculum is known for better tolerance of ammonia and total volatile fatty acid (TVFA) levels so that performance of AD can be improved as feeding with various wastes (Abouelenien et al.,2009; Güngör-Demirci et al.,2004; Woon et al.,2012a).Unlike the AD of WAS and biosolids, there are limited numbers of anaerobic digesters being operated with meat industry waste, it is difficult to start-up the reactor of DAF sludge with inoculum from an existing reactor.

Thus, RMIT lab maintains a mesophilic reactor feeding of DAF wastewater (Figure 5-1) for more than one year, effluent was taken out from this reactor and used as acclimated inoculum for DAF sludge reactors. Before the AD experiment carried out, the acclimated inoculum were characterised and the results shown in Table 5-7.

Table 5-7: Characteristics of the anaerobic acclimated inoculum used in this study

Experiment:	Mesophilic AD of DAF sludge of low TS with acclimated inoculum	
Parameters	(Unit)	date: 28/9/2012
TS	mg/L	6,230
VS	mg/L	4,755
pH	pH	8.12
CODt	mg O_2/L	17,560
CODs	mg O_2/L	3,800
TP	mg P/L	90
TN	mg N/L	680
TAN	mg N/L	362.0
TVFAs	(mg AceticAcid/L)	1500

5.2.Analytical Techniques

5.2.1. Biogas and Methane Measurements

The biogas produced was measured daily though a water displacement unit at room temperature of 1 atm. Methane content was measured. Methane content was determined according to the method used by (Demirer et al.,2008), in which a known volume of the gas sample taken from a reactor, was syringed out and injected into another serum bottle containing 20 g/L Potassium hydroxide solution. The serum bottle was then shaken manually for 3-4 min to allow all the CO_2 and H_2S to be absorbed into the KOH (potassium hydroxide) solution. The ratio of remaining gas volume (CH_4 and H_2) and the initial injected volume gave the content of CH_4 in the biogas sample, and can be calculated as the Equation 5-1 below:

$$\boldsymbol{methane\ content}\ (\%) = \frac{\boldsymbol{ammount\ of\ gas\ remained}}{\boldsymbol{amount\ of\ gas\ injectied}} \times \mathbf{100}\% \qquad \textbf{Equation 5-1}$$

5.2.2. **TS and VS**

The total solids (TS) and total violated solids (TVS) were measured according to the Standard Method procedure 2540G (APHA,2005).

The TS or VS reduction was calculated in the following Equation 5-2:

$$\boldsymbol{solids\ reduction}\ (\%) = \frac{\boldsymbol{Solids\ content\ in\ the\ feed - Solids\ content\ in\ the\ effluent}}{\boldsymbol{Solids\ content\ in\ the\ feed}} \times \mathbf{100}\% \quad \textbf{Equation 5-2}$$

5.2.3. **COD, TP, TN, TAN, TVFA and pH**

The pH of the waste and the effluent was measured by the Mettler Toledo® SevenEasy pH meter.

The chemical parameters (COD, TN, TP, TAN and TVFAs,) were measured through colorimetric techniques, using a HACH spectropotometer (DR5000). For the determination of soluble parameters, such as CODs, TAN and TVFAs, samples were centrifuged then filtered using 0.45µm nitrocelleulose membrane filters (Millipore CAS #9004-70-0) before testing.

HACH test kits were used to measure the concentration of COD, TAN, TP, TN and TVFAs. COD was measured using the HACH method 8000 (TNT/COD UHR). Ammonia was measured using the HACH method 10031 (Reagent Set, High Range Test 'N Tube™ AmVer™ nitrogen ammonia). Total phosphorus measurement used the HACH method 10127 (Test 'N Tube™ Reagent Set- high range molybdovanadate). Total nitrogen followed the HACH method 10072 (Total nitrogen Reagent Set, HR, TNT). TVFA was measured using the HACH method 8196 (TVFAs Reagent Set, Esterification).

The COD reduction was calculated as the following Equation 5-3:

$$\mathbf{COD\ reduction}\ (\%) = \frac{\mathbf{CODt\ in\ the\ feed - CODt\ in\ the\ effluent}}{\mathbf{CODt\ in\ the\ feed}} \times \mathbf{100}\% \quad \textbf{Equation 5-3}$$

5.2.4. **Digestate Quality**

The settling property of the AD digested sludge was investigated by allowing the effluent from the reactors to settle by gravity for four hours. Photos were taken to assess the amount of sludge settling by gravity.

5.2.5. **Microbiological test**

Microbiological tests assessed the remaining pathogens level in the digested sludge, which determines the class of the digestate and its potential beneficial uses. Samples were collected from reactors and kept in sterilised containers with ice, before being sent to a commercial lab. Microbiological tests were conducted within 24 hours of samples being collected from digesters by the ALS group, a testing services provider.

5.3.Experimental Procedures

5.3.1. **Batch Experiments**

BMP tests were performed in batch mode. Batch tests have been well used in research because they are easy to conduct, time efficient and economic. Batch reactors were filled with anaerobic inoculum and substrate at certain ratios on day one, and kept in incubators of a designed temperature range. The amount of biogas was measured over time, and the effluent of the reactor was tested at the end of experiment as the indication of its supernatant and sludge quality. In batch tests, organic loading (OL) refers to the ratio of total COD (CODt) mass in the substrate to the total mass of VS in the reactors (as mixed with inoculum and substrate).

5.3.1.1. *BMP from WAS*

The experiment was carried out using 250 mL serum bottles, which were fed with inoculum and biosolids slurry at OL of 0.59 and 0.24 gCOD/gVS. The effective volume of each reactor was 100 mL. All batch reactors ran in duplicates and were placed in an incubator at 100 rpm and 35±2ºC. The biosolids slurry and anaerobic raw inoculum was characterised before the test, and results can be found in Table 5-1 and Table 5-6.

The biogas produced in each reactor was measured daily though a water displacement unit at a room temperature of 1 atm. Weekly testing of biogas methane content was conducted. The pH, CODt, soluble COD (CODs), TAN, TS and VS and TVFA of the effluents from reactors were measured on day 35.

5.3.1.2. *BMP from Biosolids*

The experiment was carried out using 250 mL serum bottles, which were fed with inoculum and biosolids slurry at OL of 0.59 and 0.24 gCOD/gVS. The effective volume of each reactor was 100 mL. All batch reactors ran in duplicates and were placed in an incubator at 100 rpm and 35±2ºC. The biosolids slurry and anaerobic raw inoculum was characterised before the test, and results can be found in Table 5-2 and Table 5-6.

The biogas produced in each reactor was measured daily though a water displacement unit at a room temperature of 1 atm. Weekly testing of biogas methane content was conducted. The pH, CODt, CODs, TAN, TS and VS and TVFA of the effluents from reactors were measured on day 35.

5.3.1.3. *BMP from Codigestion of Biosolids and DAF sludge*

The ratio of the biosolids slurry and DAF sludge feeding into the reactors was selected as 2:3 v/v based on the daily production rate of the ATP and the meat industry. Characteristics of the DAF sludge and biosolids mixture is shown below.

Table 5-8: Characteristics of the Mixture of DAF Sludge and Biosolids Slurry

Experiment:	BMP from DAF sludge and Biosolids	
Parameters	(Unit)	date:9/9/2011
TS	mg/L	39000
VS	mg/L	33000
pH	pH	6.42
CODt	mg O_2/L	76000
CODs	mg O_2/L	6700
TP	mg P/L	3000
TN	mg N/L	3600
TAN	mg N/L	580
TVFAs	(mg AceticAcid/L)	1300

The experiment was carried out using 250 mL serum bottles, which were fed with inoculum and WAS at OL of 1.29 and 0.55 gCOD/gVS. The effective volume of each reactor was 100 mL. All batch reactors ran in duplicates and were placed in an incubator at 100 rpm and 35±2ºC. The raw inoculum was characterised before the test, and results can be found in Table 5-6. The pH, CODt, CODs, TAN, TS and VS and TVFA of the effluents from reactors were measured on day 170170.

5.3.2. Semi-continuous Tests (Mesophilic AD and TPAD)

Batch tests are easy to conduct, less time consuming and more economic; however, semi-continuous experiments can simulate the real situation of the AD. For semi-continuous conditions, the reactors were wasted and filled with substrate every day, thus the results give a better indication of biogas production rates and effluent quality.

In semi-continuous tests, OLR can be expressed as g VS/L.day, which is the mass of VS in the daily feeding substrate to the volume of the AD reactors. HRT is time that the liquid sludge is kept in the reactor, whereas the SRT is the average time solids stay in the digester. The experiments in this paper ran in the complete mixed system, where liquid and solids/biomass mixed together all the time, thus SRT and HRT were the same.

5.3.2.1. *Mesophilic AD and TPAD of thickened WAS (Semi-continuous condition)*

The mesophilic AD and TPAD from the WAS experiment was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic raw inoculum and thickened WAS sample are shown in Table 5-6 and Table 5-1 respectively.

Thickened WAS to anaerobic inoculum ratios were according to the HRT on day one and onwards. Reactors were placed in shakers at 100 rpm. The mesophilic AD and second phase AD reactors (of the TPAD system) were kept at 35±2°C, whereas the first phase reactors of TPAD system were kept at 55±2°C.

Mesophilic reactors were operated at a HRT of 14 and 23 days, labelled as WM14 and WM23, respectively. The first phase TPAD reactors were operated at a HRT of four and six days (labelled as WT4 and WT6), and the effluent from first phase reactors were fed into the corresponding second phase reactors for another 10 days (labelled as WTP10) and 17 days (labelled as WTP17), respectively. A summary of the experimental setup is given in Table 5-9. All the reactors ran in duplicates.

Table 5-9: Experiment setup of Mesophilic AD and TPAD from WAS

Reactor Label	Temperature	HRT (days)	Loading Rate (g VS/L.day)
WM14	35°C	14	2.2
WM23	35°C	23	1.5
WT4	55°C	4	2.2
WTP10	35°C	10	
WT6	55°C	6	1.5
WTP17	35°C	17	

5.3.2.2. *Mesophilic AD and TPAD of Biosolids*

The experiment of mesophilic AD and TPAD from biosolids was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic raw inoculum and diluted biosolids sample are shown in Table 5-6 and Table 5-2, respectively.

Diluted biosolids to anaerobic inoculum ratios were according to the HRT on day one and onwards. Reactors were placed in shakers at 100 rpm. The mesophilic AD and second phase AD reactors (of the TPAD system) were kept at 35±2°C, whereas the first phase reactors of TPAD system were kept at 55±2°C.

Mesophilic reactors were operated at a HRT of 14 and 23 days, labelled as BM14 and BM23, respectively. The first phase reactors were operated at a HRT of two days (labelled as BT2), and the effluent from first phase reactors were fed into the second phase reactors for another 10 days (labelled as BTP12) and 21 days (labelled as WTP21), respectively.

A summary of the experimental setup is given in Table 5-10. All the reactors ran in duplicates.

Table 5-10: Experiment Setup of Mesophilic AD and TPAD of Biosolids

Reactor Label	Temperature	HRT (days)	Loading Rate (g VS/L.day)
BM14	35°C	14	0.94
BM23	35°C	23	0.57
BT2	55°C	2	0.94
BTP12	35°C	12	
BT2	55°C	2	0.57
BTP21	35°C	21	

5.3.2.3. *Mesophilic AD and TPAD of DAF sludge*

5.3.2.3.1. Raw DAF Sludge

The experiment of mesophilic AD and TPAD from raw DAF sludge was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic raw inoculum and DAF sludge sample are shown in Table 5-6 and Table 5-3, respectively.

Raw DAF sludge to anaerobic inoculum ratios were according to the HRT on day one and onwards. Reactors were placed in shakers at 100 rpm. The mesophilic AD and second phase AD reactors (of the TPAD system) were kept at 35±2°C, whereas the first phase reactors of TPAD system were kept at 55±2°C.

Mesophilic reactors were operated at a HRT of 30 days, labelled as DM30. The first phase reactors were operated at a HRT of eight days (labelled as DT8), and the effluent from the first phase reactors were fed into the second phase reactors for another 15 days (labelled as DTP15) and 20 days (labelled as DTP20), respectively.

A summary of the experimental setup is given in Table 5-11. All the reactors ran in duplicates.

Table 5-11: Experiment setup of Mesophilic AD and TPAD of Biosolids

Reactor Label	Temperature	HRT (days)	Loading Rate (g VS/L.day)
DM30	35°C	30	2.66
DT8	55°C	8	3.47
DTP15	35°C	15	
BT8	55°C	8	2.85
BTP20	35°C	20	

5.3.2.3.2. DAF sludge of Low TS

The experiment of mesophilic AD from DAF sludge of low TS was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic raw inoculum are shown in Table 5-6.

In order to match the feed slurry with the recommended loading rate from US EPA(1979), DAF sludge was diluted with tap water and formed the DAF-A, DAF-B and DAF-C correspondingly, where DAF A had the highest content of DAF sludge and DAF C had the least content of DAF sludge. Characteristics of the DAF sludge of low TS are shown in Table 5-12.

Table 5-12: Characteristics of the DAF sludge of low TS in section 6.2.3 and 6.3.3

Sample day	11/3/12	1/4/12	5/13/12	26/5/12	7/6/12	5/7/12
VS	mg VS/L	mg VS/L	mg VS/L	mg VS/L	mg VS/L	mg VS/L
DAF-A	55,646	46,595	37,670		43,029	31,555
DAF-B	35,861	30,028	24,276		27,730	20,335
DAF-C	16,076	13,461	10,883		17,212	9,116
CODt	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L
DAF-A	125,345	119,045	110,455	113,291	105,443	77,325
DAF-B	80,778	76,718	71,182	73,010	67,952	49,832
DAF-C	36,211	34,391	31,909	32,728	42,177	22,338

On day one, DAF sludge of low TS to anaerobic inoculum were mixed at a ratio of 30:70 v/v, which was similar to the approach of starting batch tests, and according to the HRT onwards. Reactors were placed in shakers at 100 rpm under 35±2°C.

Reactors, fed daily with DAF A (highest organic content) and run under a HRT of 14 and 23 days, were labelled as M-14-A-daf and M23-A-23-daf, respectively. Reactors, fed daily with DAF B and run under a HRT of 14 and 23 days, were labelled as M-14-B-daf and M-B-23-daf, respectively. Similarly, reactors fed daily with DAF C (lowest organic content) and run under a HRT of 14 and 23 days, were labelled as M-14-C-daf and M-C-23-daf correspondingly. A summary of the experimental setup is given in Table 5 13. All the reactors ran in duplicates.

Table 5-13: Experiment setup of Mesophilic AD from DAF sludge of low TS

	Temperature	HRT	loading
Reactor	°C	Day	kg VS /m^3
M-14-DAF-A	35°C	14	3.9
M-14-DAF-B	35°C	14	2.6
M-14-DAF-C	35°C	14	1.2
M-23-DAF-A	35°C	23	3.2
M-23-DAF-B	35°C	23	1.5
M-23-DAF-C	35°C	23	0.7

5.3.2.3.3. Non-polymer DAF Sludge

The non-polymer DAF sludge here refers to the concentration of the DAF wastewater by centrifuge without polymers. The experiment of mesophilic AD and TPAD from non-polymer DAF sludge was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic raw inoculum and non-polymer DAF sludge sample are shown in Table 5-6 and Table 5-5, respectively.

Non-polymer DAF sludge to anaerobic inoculum ratios were according to the HRT on day one and onwards in terms of the reactors starting up. Reactors were placed in shakers at 100 rpm to provide adequate mixing. The mesophilic and second phase AD reactors (of the TPAD system) were kept at 35±2°C, whereas the first phase reactors of TPAD system were kept at 55±2°C.

Mesophilic reactors were operated at a HRT of 23 days, labelled as NM23. The first phase reactors were operated at a HRT of four and six days (labelled as NT4 and NT6), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as NTP10) and 17 days (labelled as NTP17), respectively.

A summary of the experimental setup is given in Table 5-14. All the reactors ran in duplicates.

Table 5-14: Experiment Setup of Mesophilic AD and TPAD of Non-polymer DAF Sludge

Reactor Label	Temperature	HRT (days)	Loading Rate (g VS/L.day)
NM23	35°C	23	0.62
NT4	55°C	4	1.01
NTP10	35°C	10	
NT6	55°C	6	0.62
NTP17	35°C	17	

5.3.2.3.4. With Acclimated Inoculum

The experiment of mesophilic AD from DAF sludge of low TS with acclimated inoculum was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic inoculum, acclimated inoculum and DAF sludge of low TS are shown in Table 5-6, Table 5-5 and

Table 5-15, respectively. As a comparison with the acclimated inoculum, another set of reactors running at exactly the same conditions and the same feeding of DAF sludge but with anaerobic raw inoculum (characteristics shown in Table 5-6) were also conducted. Reactors were all placed in shakers at 100 rpm under 35±2°C.

Table 5-15: Characteristics of the DAF Sludge of Low TS for Mesophilic AD with Acclimated Inoculum

		Before Mixing	After Mixing with Water
Parameters	Unit	11/3/12	11/3/12
TS	mg/L	66,049	19,815
VS	mg/L	58,868	17,660
pH	pH	6.16	6.16
CODt	mg O_2/L	157,800	47,340
CODs	mg O_2/L	9,040	2,712
TN	mg N/L	2080	624
TAN	mg N/L	452	136.0
TVFAs	mg AceticAcid/L	3,292	988

Reactors started with the substrate to acclimated inoculum ratio according to HRT on day one and onwards, ran under a HRT of 23 and 30 days and were labelled as HM23-acc and HM30-acc, respectively. Reactors started with the substrate to raw inoculum (non-acclimated inoculum) ratio according to HRT on day one and onwards, ran under a HRT of 23 and 30 days and were labelled as HM23-non and HM30-non, respectively.

A summary of the experimental setup is given in Table 5-16. All the reactors ran in duplicates.

Table 5-16: Experiment Setup of Mesophilic AD of DAF Sludge of Low TS with Acclimated Inoculum

Reactor Label	Temperature (°C)	HRT (days)	Loading Rate (g VS/L.day)	Inoculum type
HM23-acc	35	23	0.58	Acclimated
HM23-non	35	23	0.44	Sludge (non-acclimated)
HM30-acc	35	30	0.58	Acclimated
HM30-non	35	30	0.44	Sludge (non-acclimated)

5.3.2.4. *Mesophilic and TPAD Codigestion of WAS and DAF Sludge*

The experiment of mesophilic AD and TPAD from codigestion of WAS and DAF sludge was carried out under semi-continuous conditions, where all reactors were fed once on a daily basis. Characteristics of the anaerobic inoculum, DAF sludge and WAS sample are shown in Table 5-6 , Table 5-3 and Table 5-1 respectively.

In order to compare the results with the mesophilic AD of DAF sludge of low TS, this experiment is designed with similar conditions. WAS and DAF sludge were mixed to form the similar VS content of DAF-A, DAF-B and DAF-C, which were then named as slurry A, slurry B and slurry C severally. Characteristics of the DAF and WAS mixtures are shown on Table 5-17.

Table 5-17: Characteristics of the DAF sludge and WAS mixture for mesophilic AD and TPAD from codigestion of WAS and DAF sludge

Sample day	11/3/12	1/4/12	5/13/12	26/5/12	7/6/12	5/7/12
VS	mgVS/L	mgVS/L	mgVS/L	mgVS/L	mgVS/L	mgVS/L
slurry A	57,186	53,895	44,077		51,200	52,450
slurry B	37,477	37,530	30,985		33,500	32,050
slurry C	17,767	15,710	13,528		14,100	12,530
CODt	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L	mg O_2/L
slurry A	126,376	133,205	123,755	130,164	143,625	134,592
slurry B	81,370	90,170	83,870	91,100	99,750	95,472
slurry C	36,364	32,790	30,690	307,00	43,500	34,728

Under mesophilic conditions, reactors fed with slurry A run under a HRT of 14 and 23 days are labelled as M-14-A and M-23-A, respectively. Reactors fed with slurry B run under a HRT of 14 and 23 days are labelled as M-14-B and M-23-B correspondingly. Reactors fed with slurry C run under a HRT of 14 and 23 days are labelled as M-14-C and M-23-C, respectively.

All the mesophilic reactors were run at a HRT of 14 days and 23 days, whereas TPAD reactors were run at the first phase (55◦C) for four and six days, and then fed into the second phase reactors (35◦C) for another 10 days and 17 days, respectively. By this setup, the TPAD reactors had the same total HRT as the mesophilic AD reactors.

The first phase reactors fed with slurry A were operated at a HRT of four and six days (labelled as T-4-A and T-6-A), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-A) and 17 days (labelled as TP-17-A), respectively. The first phase reactors fed with slurry B were operated at a HRT of four and six days (labelled as T-4-B and T-6-B), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-B) and 17 days (labelled as TP-17-B), respectively. The first phase reactors fed with slurry C were operated at a HRT of four and six days (labelled as T-4-C and T-6-C), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-C) and 17 days (labelled as TP-17-C), respectively.

All reactors were run in duplicates and placed in shakers at 100 rpm under 35±2◦C for the mesophilic and second phase TPAD, and under 55±2◦C for the first phase TPAD. A summary of the experimental setup is given in Table 5-18.

Table 5-18: Experiment Setup of Mesophilic AD and TPAD from Non-polymer DAF Sludge

Reactor Label	Temperature	HRT (days)	Loading Rate (g VS/L.day)
M-14-A	35°C	14	3.85
M-14-B	35°C	14	2.51
M-14-C	35°C	14	1.05
M-23-A	35°C	23	2.34
M-23-B	35°C	23	1.53
M-23-C	35°C	23	0.64
T1-4-A	55°C	4	3.85
T2-10-A	35°C	10	
T1-4-B	55°C	4	2.51
T2-10-B	35°C	10	
T1-4-C	55°C	4	1.05
T2-10-C	35°C	10	
T1-6-A	55°C	6	2.34
T2-17-A	35°C	17	
T1-6-B	55°C	6	1.53
T2-17-B	35°C	17	
T1-6-C	55°C	6	0.64
T2-17-C	35°C	17	

Anaerobic operation temperature is higher than aerobic digestion therefore the energy consumption could be higher. It is necessary to look at the energy potential and compare the possible energy consumption. Detailed discussion and calculation can be found in section 7. Based on the theoretical calculation, anaerobic sludge digestion has a positive energy net gain.

6. Results and Discussion

AD is an efficient technology for energy recovery from the sludge generated from wastewater treatment (see section 3.3). The potential for energy recovery from sludge using AD can be estimated based on biogas or methane yield.

The aim of this section was to investigate the BPP of the sludge generated from wastewater treatment through batch tests, and also to evaluate the performance of semi-continuous anaerobic digesters under different operation conditions.

WAS samples used in the tests were collected after the IDEA tanks and biosolids samples used in this test were collected from the ATP. These solids were the aerated sludge after being dewatered using BFP. The process of ATP flowchart is shown in Figure 2.1. Though the Biosolids had been treated using an aerobic digestion system, the biosolids had to be transported off site for further treatment. The aims of tests for both WAS and biosolids were to compare their biogas and methane potential (i.e., before and after aerobic digestion) and to look into the feasibility of sending these solids to AD rather than composting.

DAF sludge was also tested as its high organic content can boost the organic carbon source for the AD reactors. A sample was collected from a dissolve air floating system in a local meat industry facility.

6.1 Mesophilic AD in batch mode/experiments (BMP)

BMP laboratory batch tests are used to estimate the methane yield from a given waste material. Batch tests have been widely used in the area of AD research, because it is easy to carry out, is time efficient and economic. The procedure followed in this study to carry out BMP is outlined in section 5.3.1.

This section provides a discussion of the results from the AD batch tests for WAS, biosolids and biosolids and DAF. The effectiveness of AD for these materials was assessed based on biogas yield and TS and VS reduction..

6.1.1. **BMP from WAS**

BMP from WAS at different OLs is shown in Figure 6-1. The biogas production rate showed a similar trend for all OLs (gCOD waste/gVS). The biogas production rate during the first seven days was the fastest, but the rate continued to decrease for the remainder of the test duration. This trend indicates that there was more organic source available for the anaerobic organisms to biodegrade and converts into biogas at the beginning of the BMP test. The fraction of easily biodegrade organics in simple form, such as sugar, was converted into biogas at the early stage of the AD period. In different loadings, reactors with higher loading contained more of that simple organic fraction compared with lower loading reactors at the beginning. The fraction of more complex organics had been degraded into the simpler form and then continued to be converted into biogas, which took longer than the conversion of biogas from simple organics. As digestion continued, less and less simple organic substrate was available for biodegradation; this was reflected in the lower rate of biogas production.

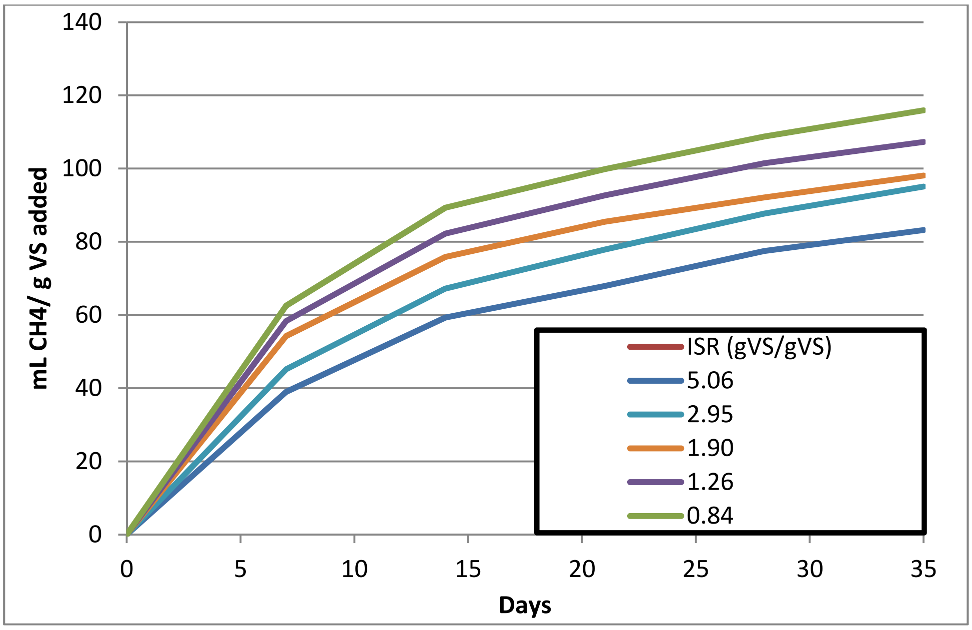


Figure 6-1: Accumulated Methane from WAS using BMP

The biogas yield from WAS, under batch conditions, showed a proportional relationship with the OL applied. In these batch tests, the OL refers to the ratio of CODt of the substrate to the total mass of VS in the reactors. The mass of CODt is a measure of the organic matter for anaerobic biodegradation (Ratnayaka et al.,2009). Higher loading means higher amounts of organics being degraded in a given anaerobic population in a given reactor volume. Hence, the aim of assessing BMP for the waste at different loadings is to identify the ratio of organics to degradable solids (VS) in the reactor at which the highest yield is obtained.

The reactor that received the highest loading of 0.71 gCOD/gVS produced the highest accumulative volume of biogas of 183mL/g VS (116 mL methane/gVS), measured after 35 days. The accumulative methane yield showed a liner relationship (R^2 value of 0.977) with the loading (see Figure 6-2). R^2 value here indicates how linear the trend is; when it equals to 1 it means the trend is absolute linear. The tested condition was under loaded that how the linear relationship occurred. In the literatures, the loading rate has the optimum range which means the liner relationship only exists in certain range. However, the under loaded conditions were not reported as industry will be only interested in high loading reactors where operation is much more feasible. Hence there is no literature to be compared in this case.

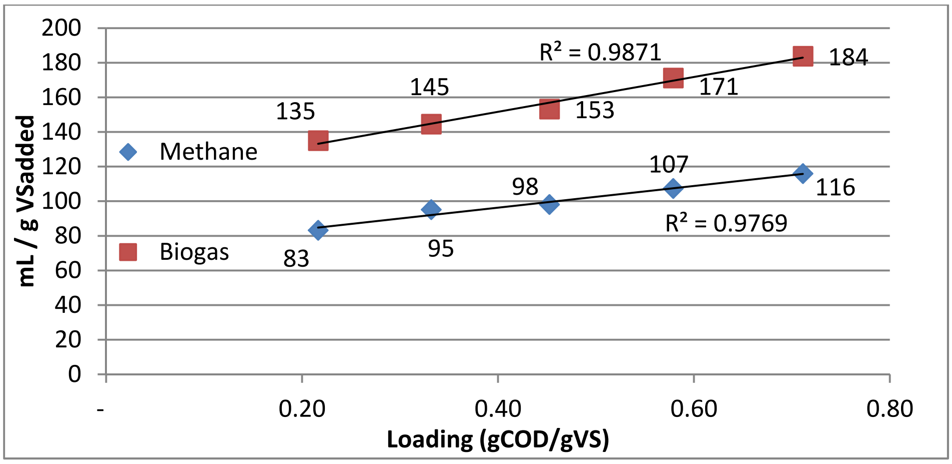


Figure 6-2: Accumulated methane verse loading (BMP of WAS)

In order to assess the effectiveness of AD for a given substrate all reactors were tested for CODt and VS before and after digestion. The highest CODt removal of 36% was obtained in the reactor that received the highest OL (0.71 gCOD/gVS) (see Figure 6-3). Similarly, the highest VS removal of 30% was obtained from the same reactor.

In reactors with lower OLs, the ratio of CODt and VS contributed by the inoculum was higher, which is less biodegradable comparedwith the CODt and VS from the WAS. Hence, these reactors had lower CODt and VS removal. In the reactors of loading at 0.22 to 0.45 gCOD/gVS, the ratios of CODt and VS from WAS were less significant compared with the inoculum, and the removal rates were almost the same.

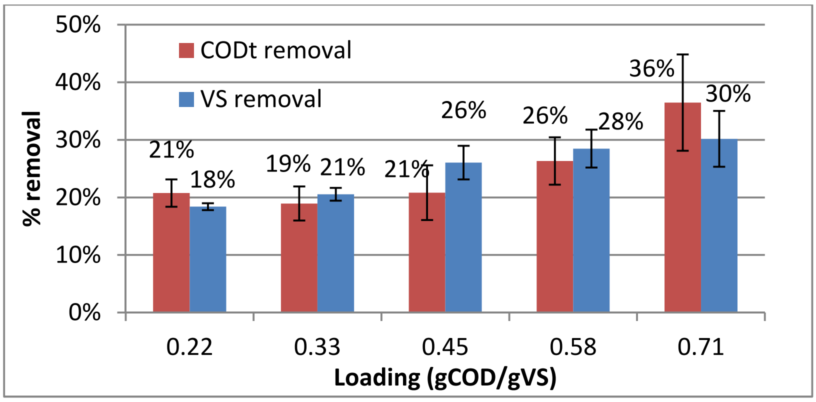


Figure 6-3: Organic removal in AD of WAS batch test

During the AD process, ammonia is the by-product from the biodegradation of nitrogenous matter, which is usually present in the feedstock in the form of proteins and urea. The concentration of ammonia in the reactors is shown in Figure 6-4. Ammonia is known to have an inhibitory effect on AD process if its concentration in the AD reactor exceeds 1,500 mg N/L (Gerardi,2003). The highest concentration of ammonia in the reactors was 924 mgN/L in reactor under the loading of 0.33 gCOD/gVS, which is lower than the reported threshold concentration for inhibition. As mentioned before, the highest methane yield and best organic removal was the reactor at the highest loading of 0.77 gCOD/gVS. Theoretically, the reactor at loading 0.77 gCOD/gVS should have the highest ammonia concentration. That is because the batch reactors received different amounts of inoculum and waste substrate on day one, which resulted in different initial ammonia concentrations in reactors. The inoculum initial ammonia concentration was 804 mg N/L compared with 1.4 mg N/L for WAS. The reactors at low loading received more inoculum than those at high loadings; hence the initial ammonia concentration in the reactors at low OLs was less than at high loadings. Looking at the change in ammonia concentration in the reactors during the AD process, it was observed that ammonia generation in these reactors was proportional to the amount of waste applied. For instance, ammonia increased by 341 mgN/L in the reactor at organic loading of 0.77 gCOD/gVS compared with 254 mg N/L in reactor at OL of 0.22 gCOD/gVS.

The level of pH inside the reactors is an important parameter, since slight pH change can adversely affect the anaerobic process. The pH of WAS and anaerobic inoculum were 6.68 and 7.31, respectively. The initial pH in each reactor was measured immediately after the anaerobic inoculum and WAS were mixed; the pH value ranged from 7.18 to 7.27. The changes of pH from its initial value to the values shown in Figure 6-4 can be due to the release of TVFA from the degradation of organic matters. During anaerobic digestion period, the pH changed and the range became 6.88 to 7.07, which was within the range for efficient AD.

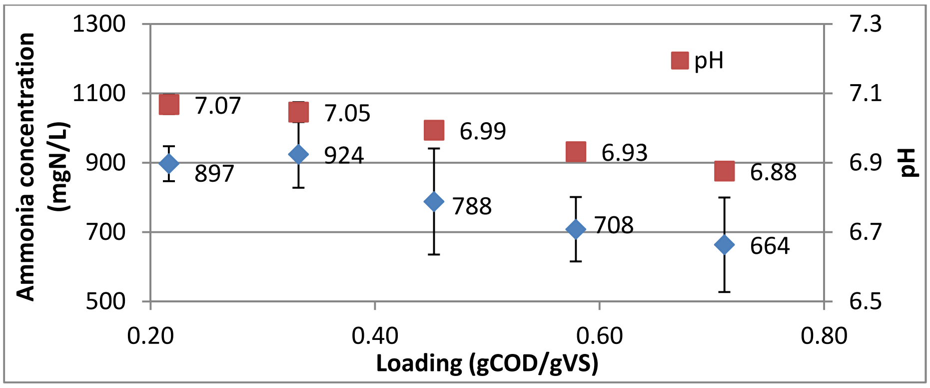


Figure 6-4: TAN and pH in the reactors receiving WAS for BMP tests.

Table 6-1: Summary of Experimental Results of BMP for WAS

ISR g VS/g VS	Loading g VS/L	Loading g COD/g VS	Biogas Yield mL/g VS_{added}	Methane Yield mL/g VS_{added}	COD Removal %	VS Removal %
5.06	2.1	0.22	135	83	21%	18%
2.95	3.2	0.33	145	95	19%	21%
1.90	4.2	0.45	153	98	21%	26%
1.26	5.2	0.58	171	107	26%	28%
0.84	6.1	0.71	184	115	36%	30%

6.1.2. **BMP from Biosolids**

Biogas yield from the dewatered biosolids ranged from 83 to 145 mL/gVS_{added} after 35 days. The higher BMP of 145 mL/gVS_{added} was found at the higher loading reactor of 0.59 gCOD/gVS by day 35, whereas the lower BMP of 83 mL/gVS_{added} was produced under the lower loading of 0.24 gCOD/gVS by day 35 (see Figure 6-5).

In order to investigate ultimate biodegradation potential for biosolids, the BMP test was extended to 170 days. An increase in biogas production in the range of 17 to 28% was obtained and resulted in cumulative biogas in the range from 106 to 170 mL/gVS_{added}.

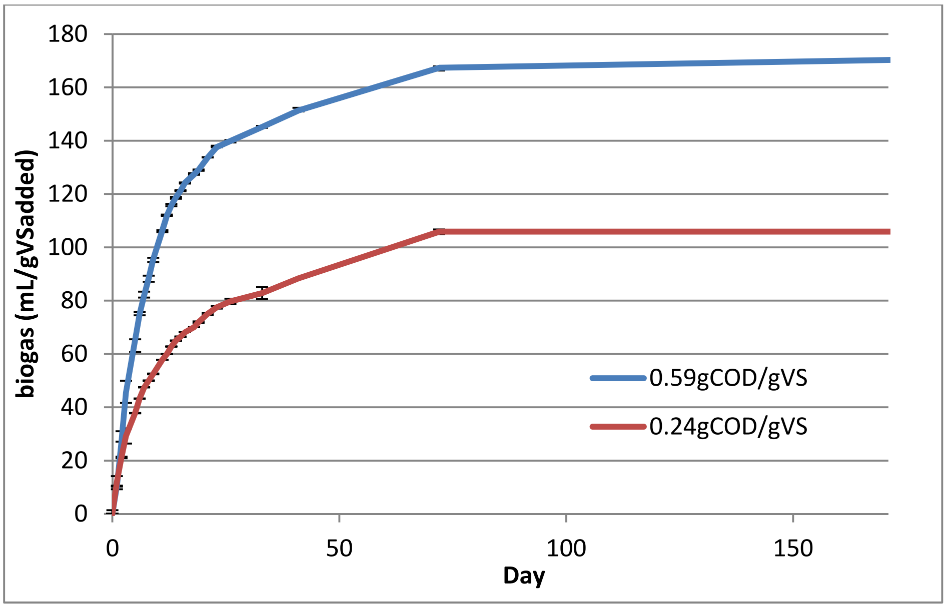


Figure 6-5: Accumulated biogas from biosolids using batch test

The concentration of organics in the reactors was measured in terms of CODt and TS. The removal of CODt and TS was 43% and 41%, respectively, after 170 days for both OLs. The retention time of the reactors was 170 days to evaluate the ultimate BMP and organic reduction. Those results showed that the ultimate removal of CODt and TS of Biosolids reached certain limits regardless of loading applied. This agrees with Rao et al. (2000), who reported the substrate remaining at infinite time is the refractory fraction or non-anaerobic biodegradable waste.

Table 6-2: Summary of Experimental Results of BMP of Biosolids

ISR g VS/ g VS	Loading gVS/L	Loading g COD/g VS	Days	Biogas	Methane	COD removal	TS removal
				mL/g VS_{added}			
1.01	2.64	0.59	35	145	89	43%	41%
			170	170	104		
4.03	2.65	0.24	35	83	51	41%	41%
			170	106	66		

6.1.3. **BMP from DAF sludge**

Woon (2012b) investigated the feasibility of biogas production from DAF sludge using batch and continuous AD reactors and reported that the biogas yield of DAF sludge ranged from 360 - 530 mL/gVS_{added} for loadings ranging from 0.83 - 3.38 gCOD/gVS. Gehesha (2012) also studied the same material with different loading rate. This study investigated the feasibility of codigestion of DAF sludge used by Woon (2012) and Gehesha (2012) with sewage sludge from ATP.

6.1.4. **BMP from Biosolids and DAF sludge**

One of the objectives in this study was to assess the BMP for individual waste and combined wastes such as WAS, DAF sludge and biosolids. As the literature states, the AD of a variety of substrates is more stable and produces better biogas yield than a single substrate (Alvarez et al.,2008; Buendia et al.,2009; Davidsson et al.,2008; Luste et al.,2010).

The biogas of DAF sludge reported by Woon (2012) was 215 mL/gVSadded, which is higher than the biogas from biosolids mentioned in section 6.1.3 under the same retention time (see Table 6-2). Adding the DAF sludge to biosolids should increase the organic matter in the biosolids slurry and produce a higher yield.

For the codigestion of DAF sludge with biosolids, the ratio of biosolids to DAF sludge was settled at 1:4 v/v (volume based ratio) based on the daily production rate of the DAF sludge and the biosolids from ATP. The codigestion reactors of DAF sludge and biosolids were assessed at loadings of 0.55 and 1.29 gCOD/gVS.

The higher biogas production of 179 mL/gVSadded was from the reactor at a loading of 1.29 gCOD/gVS, on day 35. The reactor with a lower loading of 0.55 gCOD/gVS had a lower biogas yiled of 129 mL/gVS_{added} at day 35 (see Table 6-3). The retention time of the reactors was 170 days to evaluate the ultimate BMP and organic reduction. The ultimate biogas yield from reactors was 238 mL/gVS_{added} and 139 mL/gVS_{added} from a reactor at loadings of 1.29 gCOD/gVS and 0.55 gCOD/gVS, respectively (see Table 6-3).

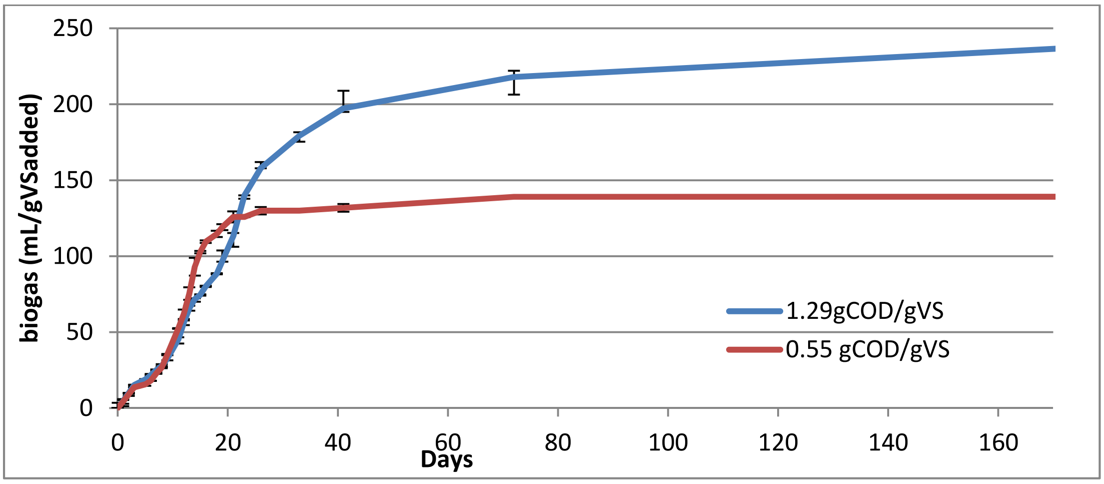


Figure 6-6: Biogas from codigestion of DAF sludge and Biosolids under batch conditions

It is noteworthy that the biogas production rate from day zero to day 23 in the reactor under loading of 0.55 gCOD/gVS was higher than that from the reactor at the loading of 1.29 gCOD/gVS. After day 23, the reactor operating at loadings of 1.29 gCOD/gVS showed higher rates of biogas production. Also, the cumulative biogas production was 179 mL/gVS$_{added}$ by day 35, which was 39% higher than production from the reactor at a loading of 0.55 gCOD/gVS. This trend is likely caused by the nature of DAF sludge, as it contains animal by-products such as blood and hair. The characteristics of DAF sludge are shown in Table 5-3. Cuetos (2008) reported a slaughterhouse waste containing 40% fat and 39% of protein. The organic from DAF sludge are not easily biodegradable hence they take longer time to solubilise and go through the degradation pathway. The reactor at a low loading received more inoculum than reactors at higher loadings, hence, was more readily biodegradable organic, and so the release of methane gas as the final stage of AD happened quicker.

In terms of the organic removal, the reactor with the higher loading of 1.29 gCOD/gVS removed 51% of TS and 60% of CODt, whereas the reactor with the lower loading of 0.55 gCOD/gVS removed 22% of TS and 36% of CODt by day 170. Unlike the AD of biosolids, when co-digesting with DAF sludge, the ultimate organic reduction showed different results with the loading. As mentioned before, the DAF sludge has more complex organic compounds; even with the reactor running for 170 days, this may not be enough time for all the biodegradable organic matter to be digested.

Table 6-3: Summary of experimental results of BMP of DAF sludge+Biosolids

ISR g VS/ g VS	Loading gVS/L	Loading g COD/g VS	Days	Biogas (mL/gVS$_{added}$)	Methane (mL/gVS$_{added}$)	COD removal	TS removal
0.8	2.93	1.29	35	179	117		
			170	238	154	60%	51%
3.3	2.73	0.55	35	129	88		
			170	139	95	36%	22%

6.1.5. **Comparison of BMP**

BMP of WAS, biosolids, DAF sludge and codigestion of DAF sludge and biosolids at 35 days are compared in Figure 6-7. Data of BMP from DAF sludge was obtained from the literature

(Woon, 2012; Gehesha,2012) because the substrate used in thestudies was collected from the same facility of DAF sludge tested here. Biogas yield from DAF sludge yielded 215 mL/gVS$_{added}$ at the loading of 2.5gCOD/gVS on day 35 (Woon et al.,2012b), which is much higher than any other substrate or mixture tested in this study if not counting the various loadings. Gehesha (2012) tested the DAF sludge under a lower loading (1.20-1.58 gCOD/gVS) by mixing the DAF sludge with tap water to the designed loading. He found that the DAF sludge yielded from 325-398 mL/gVS$_{added}$ by day 35 (see Table 6-4).

As stated earlier, the characterisation DAF sludge shows much higher organic content, that the loading of AD of DAF sludge run by Woon was much higher than this study, and even with the test done by Gehesha (2012) using diluted DAF sludge, the loading is still higher than BMP from WAS and from biosolids.

Figure 6-7 showed the comparison of BMP from WAS, DAF sludge and biosolids (after 35 days). However, if possible to exclude the effect of loading on BMP, the loading covered by this study were only found around 0.55 to 0.58 gCOD/gVS range (see Figure 6-7). Within that loading range, the order of highest BMP to lowest BMP is WAS, biosolids and then biosolids and DAF sludge.

It is interesting that the biogas yield from biosolids was only 15% lower than the BMP from WAS. The biosolids were stabilised in an aerobic sludge tank in ATP (the process chart is shown in Figure 2-1). The organic matter of the biosolids was meant to be reduced in the aeration process. However, the results here show that there are BMP for the biosolids. This might indicate that the current sludge aeration system in ATP did not achieve its designed goal to remove organic matter.

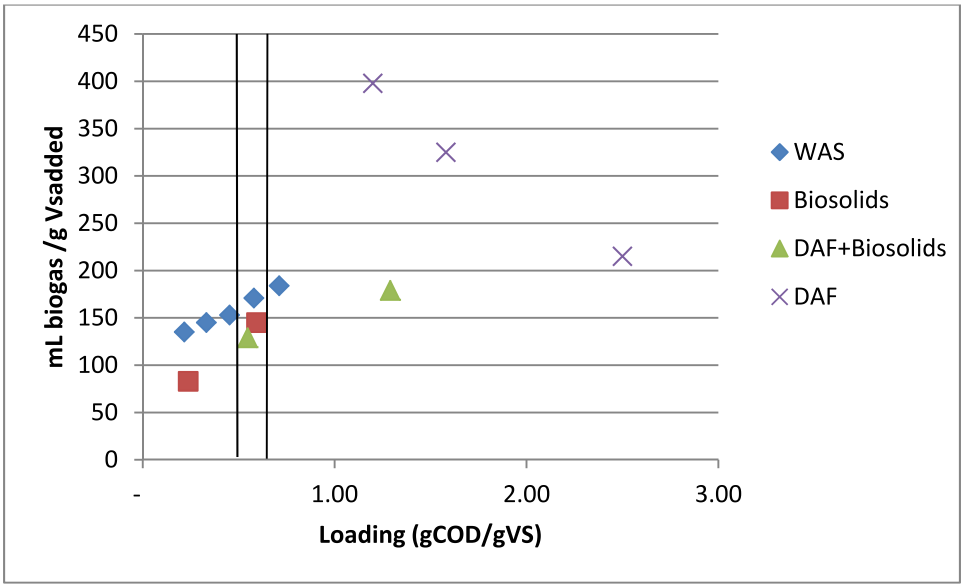


Figure 6-7: Comparison of BMP from WAS, DAF sludge and biosolids (after 35 days)

Table 6-4: Summary of Results of BMP Tests

Materials	Loading (gCOD/gVS)	Days	Biogas	Methane	COD Removal	VS Removal	Reference
			mL/gVS *				
WAS	0.22	35	135	83	21%	18%	This study
	0.33	35	145	95	19%	21%	
	0.45	35	153	98	21%	26%	
	0.58	35	171	107	26%	28%	
	0.71	35	184	115	36%	30%	
Biosolids	0.59	35	145	89	-	-	
		170	170	104	43%	41%TS	
	0.24	35	83	51	-	-	
		170	106	66	41%	41% TS	
DAF sludge+ Biosolids	1.29	35	179	117	-	-	
		170	238	154	60%	51% TS	
	0.55	35	129	88	-	-	
		170	139	95	36%	22% TS	
DAF sludge	2.50	35	215	-	-	-	Woon ,2012
DAF sludge of low TS	1.20	35	398	-	-	-	Gehesha,2013
	1.58	35	325	-	-	-	

* 100 mL/gVS = 0.1 m^3/kgVS

6.2 Mesophilic AD in semicontinuous

Batch tests are used for the screening of wastes and for an indication of the potential for biogas production from a given waste material. Once the potential is stabilised or once BMP tests show promising results, the next step would be be assess the performance of the AD reactors at different operation conditions. For semi-continuous conditions, the reactors are monitored for biogas production and they also provide data for the effluent quality and solids characteristics.

OLR can be expressed in two different means in the semi-continuous tests as gCOD/gVS (similar with the batch approach) and in g VS/L.day as based on the feeding waste only.

HRT is time that the liquid sludge is kept in the reactor, whereas the SRT is the average time solids stay in the digester. The experiments carried out as a part of this study were in the complete mixed reactors and no solids recycled, hence, SRT and HRT were the same.

6.2.1. **Mesophilic AD of WAS Under Semi-continuous Condition**

Biogas production from the mesophilic reactors that received thicken WAS of OL of 06-1.0 g VS/L.day are shown in Figure 6-8. It was observed that daily biogas production decreased during the first 35 to 45 days, labelled as period I (see Figure 6-8). This reduction indicates acclimatisation was in progress due to the daily wasting and feeding. On day one, reactors started with the substrate to inoculum ratio according to HRT. As the wasting/feeding continued, some of the inoculum (i.e., AD microorganisms) was wasted out and replaced by the thickened WAS. In the meantime, the organic fraction of WAS provided food source for the growth of new anaerobic bacteria. On average, after 35 to 45 days, the changes in daily biogas yield were less variable. This period is labelled as period II and regarded as stabilised gas production. The decreased biogas yield during the period I was also reported by Song (2004). Stabilised biogas production rates in the reactors indicate that the growth and wasting of the anaerobic microorganisms reached a balance.

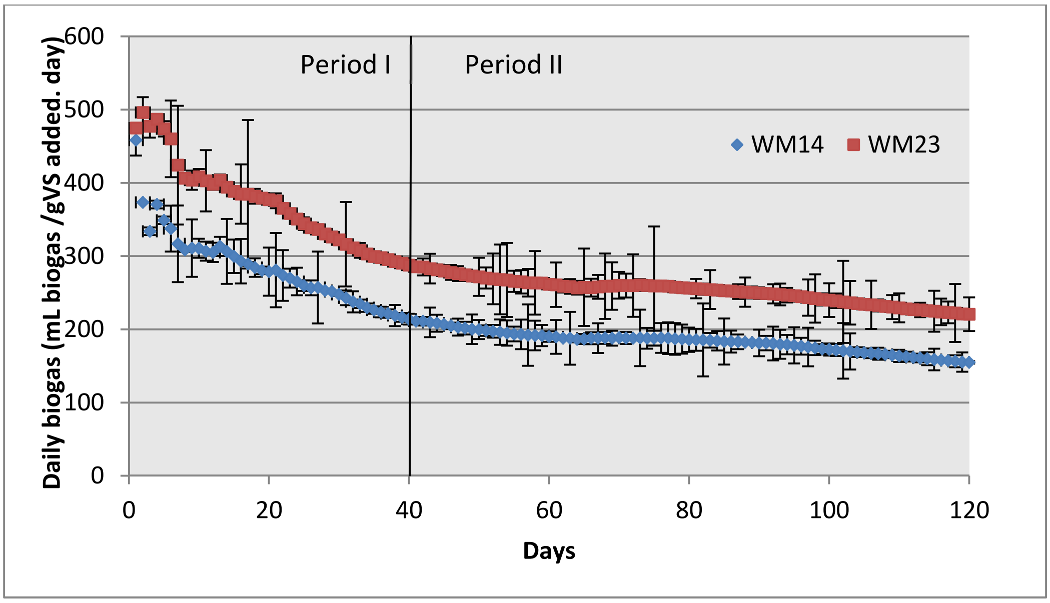


Figure 6-8: Daily biogas yield from mesophilic AD of WAS

In this mesophilic AD of WAS, the reactors operated at a HRT of 23 days were running at an OLR of 0.6 g VS/L.day, whereas the reactors operated at a HRT of 14 days were running at OLR of 1.0 g VS/L.day. The rationale behind changing both HRT and OLR was to assess the feasibility of treating a stream of substrate of consistent flow (i.e., consistent mass flow rate in gVS/day) using anaerobic digesters of different volumes. The larger the volume of the reactor or the longer the HRT results in the lower OL (in terms of g VS/L.day).

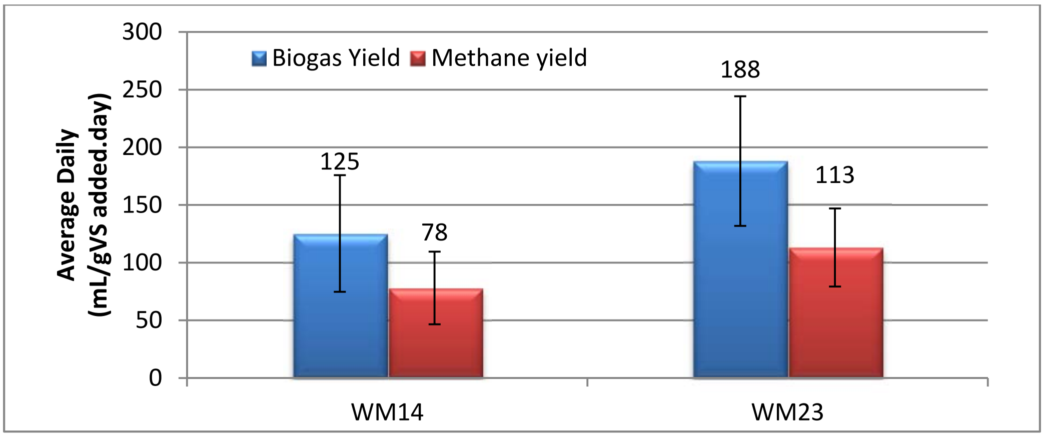


Figure 6-9: Average daily biogas and methane yield of WAS in period II

As shown in Figure 6-9, biogas yield showed a typical proportional relationship with HRT. The average daily biogas yield was 125 and 188 mL/gVS$_{added}$ at a HRT of 14 and 23 days, respectively. The biogas produced in these reactors had an average methane content of 62% and 60%, respectively. Similarly, Athanasoulia et al. (2012) investigated the mesophilic AD of WAS from an IDEA system. They reported a yield of 120 to 190 mL/gVS$_{added}$ from

WAS. They also observed the proportional relationship of biogas yield with the sludge age in the wastewater treatment activated system. Hence, the theoretical value of biogas yield can be estimated based on an empirical relationship proposed by Bolzonella (2005):

$$\textbf{Biogas yield} = 0.23\, e(-0.028 SRT) \qquad \textbf{Equation 6-1}$$

where SRT refers to the sludge retention time in the wastewater treatment activated sludge system (e.g., IDEA system in ATP). As the WAS substrate used in this experiment was collected from an IDEA system operated at a SRT of 12 days, the theoretical biogas yield was 0.164 m3/kgVSadded (equivalent to 164 mL/gVSadded). Our observed result of 125 to 188 mL/gVSadded is comparable with the theoretical values.

In addition to biogas yield, the performance of the AD reactors was assessed in terms of the quality of the supernatant and the digestate (i.e., biosolids after dewatering). In the interests of the clients, these are key parameters needed for assessing the performance of AD as an alternative waste management. The quality of the supernatant was assessed according to the trade waste criteria (refers to the liquid waste as per EPA, Australia).

The pH in all the reactors during the stabilised period of operation was in between 7.2 to 7.5 (see Figure 6 10). This is below the trade waste limits of pH 6 to 10.

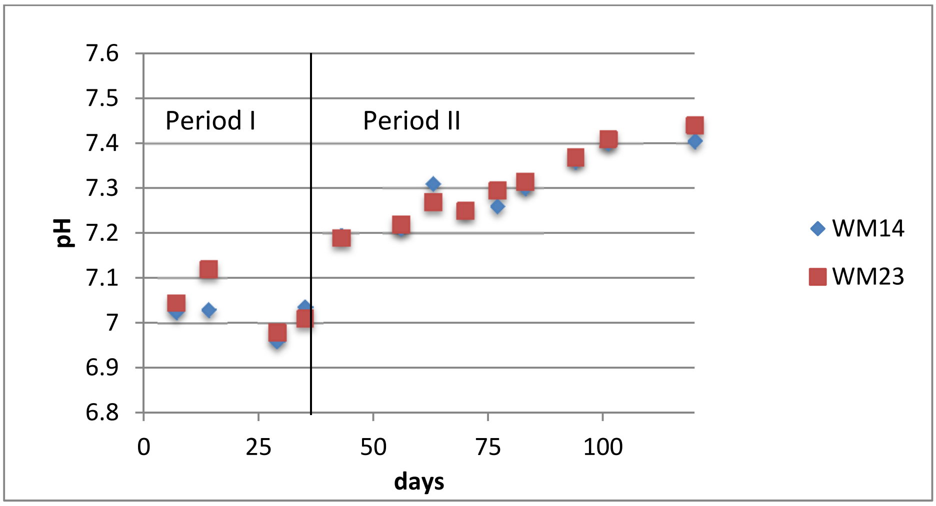


Figure 6-10: pH profile of mesophilic AD of WAS (semi-continuous)

The effluent from all the reactors had TVFAs and CODs in the range 49 to 200 and 900 to 550 mg/L, respectively, which are under the trade waste limits of 1,000 and 4,000 mg/L for TVFAs and CODs, respectively. The concentration of ammonia in the effluent from all reactors varied from 272 to 301 mgN/L, which exceeded the trade waste limit of 200 mgN/L (CWW). It is typical that ammonia level increased after AD as ammonia is the by-product from the biodegradation of nitrogenous matter in the waste, mostly in the form of proteins and urea (Chen et al.,2008).

Solids removal was monitored in in terms of TS and VS. The average VS reduction at a HRT of 14 and 23 days was 19% and 18%, respectively. The settling properties of the digested sludge were investigated by allowing the effluent from the reactors to settle by gravity for 4 hours. The sludge from both reactors showed poor settling. As shown in Figure 6-11, more than 50% of the sludge in the tube was floating after the test period of four hours. The floating sludge indicates the dispersed particles or fine particles of the gravity settling is not adequate. In light of this, polymer can be used to allow coagulation of particles for the dewatering process.

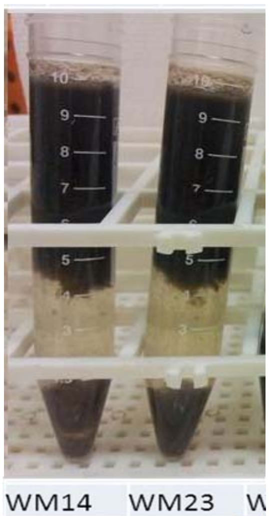


Figure 6-11: Digested WAS after four hours settling.

Table 6-5: Summary of Experimental Results from Mesophilic AD of WAS

Reactors	HRT day	OLRs gCOD/gVS	OLRs g VS/L.day	Biogas mL/gVS_{added}.day	Methane mL/gVS_{added}.day	COD Removal	VS Removal
WM14	14d	0.2	1.0	125	78	17%	19%
WM23	23d	0.1	0.6	188	133	22%	18%

6.2.2. Mesophilic AD of Biosolids Under Semi-continuous Condition

Biosolids generated at the ATP were assessed for potential usage as a source for bioenergy production. The sludge generated is treated using aerobic digestion. The digestate is dewatered using a belt press filter (BPF) and the dewatered biosolids are transported off site for further treatment. Though the WAS generated at ATP is treated in an aerobic system, the BMP batch tests showed that biosolids remaining after aerobic digestion (biosolids) are not fully stabilised and need to be further degraded for beneficial use onsite or off site. Therefore, one of the alternative waste management options at ATP was to assess the potential of AD for biosolids treatment. In addition, investigation on BMP from WAS is required to assess the need for AD and compare that with the option of AD of biosolids since it is less likely that aerobic and anaerobic digestion would both be used at the same site for WAS treatment.

According to the literature completely mixed AD is not efficient for a TS concentration of more than 10% (Battistoni et al.,2001a; Battistoni et al.,2001b; Battistoni et al.,1998; Fantozzi et al.,2011; Pavan et al.,2000; Pavan et al.,1994; Pavan et al.,1998). Hence the biosolids sample collected from ATP was mixed with tap water to the designated TS concentration before being fed into the AD reactors. In order to compare the BMP from biosolids and thickened WAS, both samples were prepared such that the VS concentration was similar. VS rather than TS was used in this case because the OLR applied was in terms of g VS/L.day. Characteristics of the WAS and biosolids used in this experiment are given in Table 5-1 and Table 5-2. The experiment procedure is discussed in section 5.3.2.2.

The daily biogas production from the biosolids both at a HRT of 14 and 23 days showed stabilised production almost after 25 days (see Figure 6-12). The start-up period was not included when calculating the average yield. .

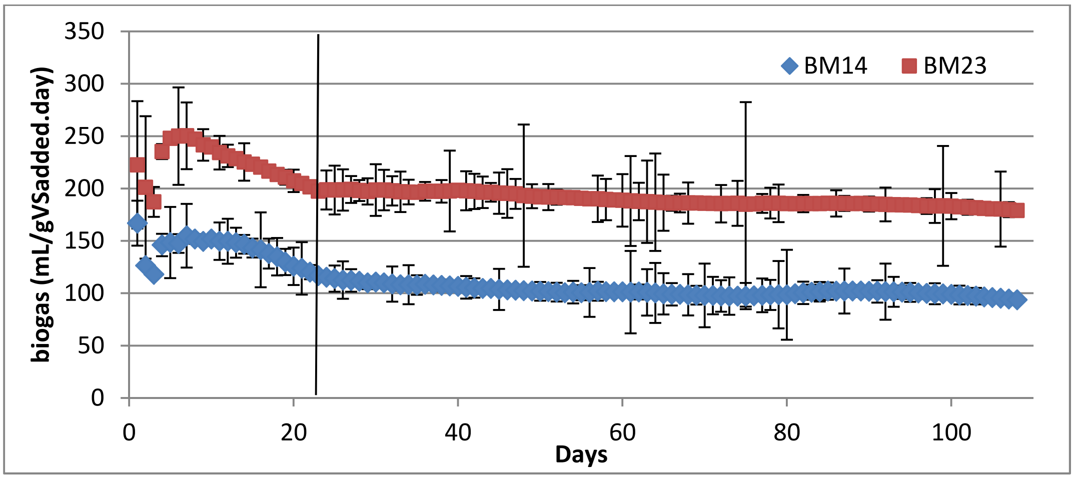


Figure 6-12: Daily biogas yield from mesophilic AD of biosolids

The reactor (BM23) operated at a HRT of 23 days (OLR of 0.6 g VS/L.day.day) produced 189 mL/gVS$_{added}$ (see Figure 6-13). This yield is 85% higher than at HRT of 14 days BM14 at OLR of 0.9 g VS/L.day. The higher yield at the OLR of 0.6 g VS/L.day indicated that longer HRT had a positive effect on the biogas yield, under the conditions tested. It is noteworthy that the effect of longer HRT on biogas yield varied between waste material. For instance, as discussed in section 6.2.1 biogas yield from WAS at a HRT of 23 days (OLR of 0.6 g VS/L.day) was 50% higher than at 14 days (OLR of 1.0 g VS/L.day).

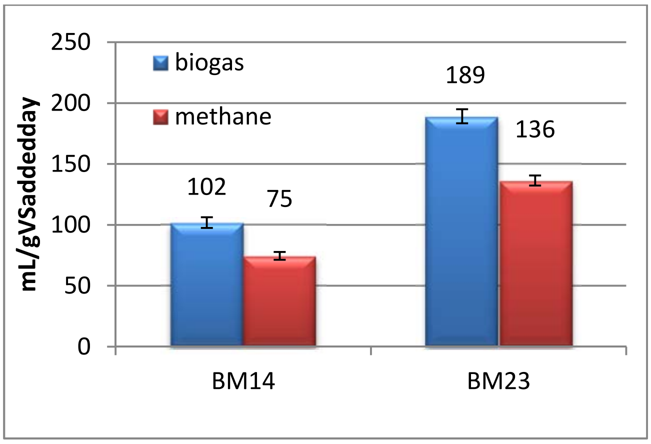


Figure 6-13: Average biogas and methane yields from mesophilic AD of biosolids over stable period

The removal of solids in terms of VS at a HRT of 14 and 23 days was 34% and 32%, respectively (see Figure 6-14). Compared with the AD of WAS, it showed 22% and 25% reduction in VS at a HRT of 14 and 23 days, respectively. That is, AD of biosolids in terms of VS removal was 10% higher than that of AD of WAS.

Other parameters for measuring the organic removal rate is the total COD, which was found to have a 35% reduction in reactor BM14 and 32% in reactor BM23. In this case, the OLR and HRT had insignificant effects on the VS and CODt reductions, whereas the biogas yields were affected greatly by changes of OLR and HRT.

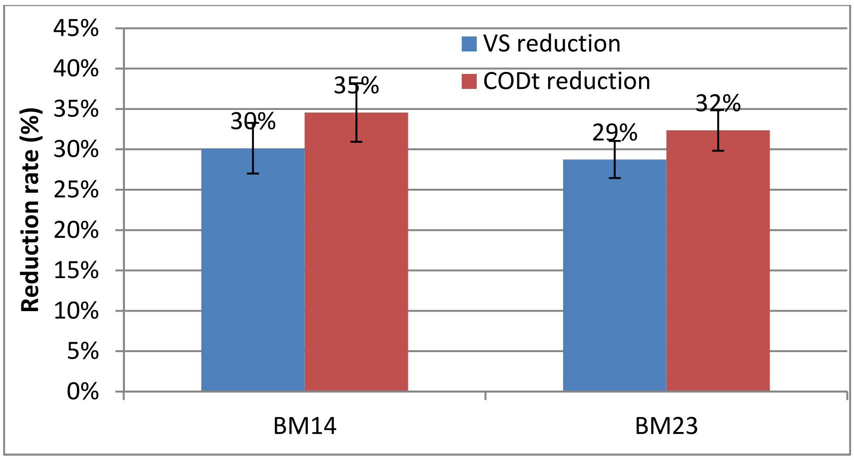


Figure 6-14: Organic reduction in the mesophilic biosolids AD reactors

Over time, the effluent from reactors had TVFAs (ranged 41-46 mgVS/L) and CODs (varied 530 to 597 mg O_2/L) concentration under the trade wastes limits of 1,000 mg TVFA/L and 4,000 mg O_2/L , respectively. The only parameter exceeding the trade waste limit of 200 mg N/L was the ammonia. Effluent from all the reactors varied from 410 to 482 mg N /L, which is excess ammonia of 210 to 282 mg N/L (see Figure 6-15).

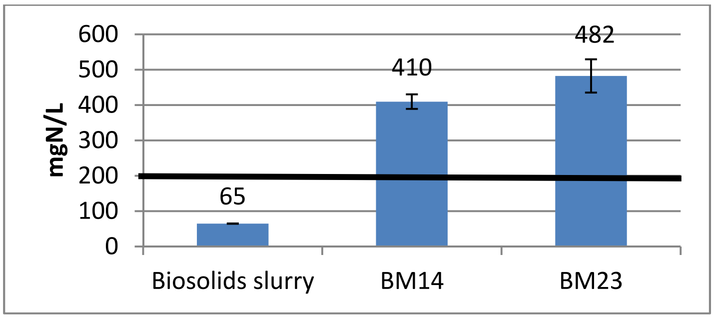


Figure 6-15 TAN in mesophilic AD of Biosolids

The digested sludge was tested for its settling property by allowing the effluent from the reactors to settle by gravity for four hours. Poorly settling sludge was found from mesophilic AD of biosolids under both loading and HRT. Compared with the digested WAS, which had more than 50%v/v of sludge floating on top of the reactor (see Figure 6-11), the digested biosolids showed very poor settling properties, No separation of solids and supernatant was observed by gravity (Figure 6-16). This can be caused by the addition of polymer in the biosolids that bond the water and solids, resulting in this hard-to-settle situation.

Figure 6-16: Digested Biosolids settling condition (after 4 hrs)

Table 6-6: Summary of Experimental Result from mesophilic AD of biosolids

Reactors	HRT	OLRs	Biogas	Methane	COD	VS

	day	gCOD/gVS	g VS/L.day	mL/gVS_{added}.day		**Removal**	**Removal**
BM14	14d	0.13	0.94	102	75	35%	30%
BM23	23d	0.08	0.57	189	136	32%	29%

6.2.3. **Mesophilic AD of DAF Sludge Under Semi-continuous Conditions**

The characteristics of DAF sludge from the meat industry showed potential for biogas production given the high concentration of COD that ranged from 141,600 mg/L to 306,400 mg/L and the high ratio of VS/TS that varied from 84% to 95% (see Table 5-3). The batch BMP tests showed that DAF sludge has good potential for bioenergy production (Woon et al.,2012a).

However, Woon (2012) also reported the semi-continuous tests of DAF sludge had low biogas yield of 26-121 mL/gVS compared with the batch result of 530 mL/gVS (see table 4-3). The DAF sludge tested by Woon was the same as received from the industry in this experiment. Woon also reported the ammonia of the digestate from the AD of DAF sludge were from 6,400 to 7,600 mg/L, which was much higher than the inhibition threshold of 1,500 mgN/L by Gerardi (2003). Hence the low biogas yield from DAF sludge under semi-continuous conditions was possibly caused by the inhibition effect of ammonia and TVFAs.

The aim of the experiments discussed in this section was to investigate the feasibility of AD of DAF sludge under semi-continuous conditions at different OLRs and HRT, for raw DAF sludge of different initial concentrations (obtained through dilution) of COD and TS. Therefore, DAF sludge was mixed with tap water to reduce COD, TS and VS, as well as the concentration of inhibiting constituents such as ammonia.

6.2.3.1. ***AD of Raw DAF sludge***

DAF sludge samples were collected from one of the meat rendering plants in Melbourne every three weeks. The sample was characterised as soon as it was received then stored at 4°C prior to use as a feedstock for the AD reactors, after mixing with water. There were variations in the characteristics of the samples collected during the experiment period. The highest TS of DAF sludge was 151 gVS/L and the lowest TS of the DAF sludge sample was 48gVS/L. Figure 6-17 shows the VS concentration of the DAF sludge samples received.

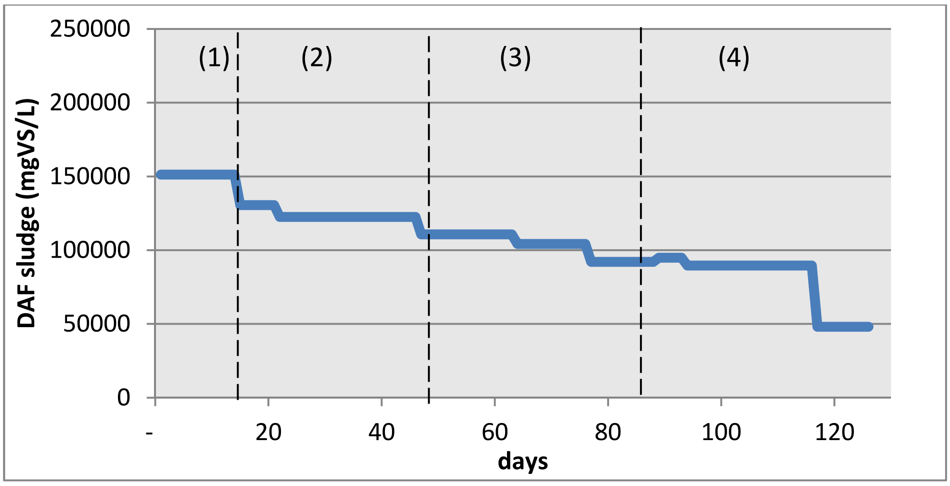


Figure 6-17: VS concentration of raw DAF sludge sample (mesophilic AD of raw DAF sludge)

The AD of raw DAF was tested under a HRT of 30 days (labelled as DM30), so that the result could provide a baseline to be compared with AD of DAF sludge of low TS in section 6.2.3.2.

The normalised daily biogas production see (Figure 6-18) shows four distinguished periods. First is the start-up period, which is similar to the AD of other substrates, when the biogas yield decreased from the beginning until around day 10. The second is the first stable period during day 11 to 44, where the standard deviations of daily biogas yield were all less than 10%. From day 45 onwards, sudden increase of standard deviations occurred until day 85. The second stable period started since then, with relatively smaller standard deviation (less than 20%). There was no significant evidence to prove the relationship between these changes of biogas yield with the variation of the VS in the DAF sludge, which is different from Woon's results (2012b). The cause of the shifting biogas yield is more likely due to the changes of dominated/majority microorganism in the AD reactors. Table 6-17 lists the average pH of the four periods. During period (1) and (2), the pH of the reactor varied from 6.25 to 6.27, which were in the optimum pH range of 5.5 to 6.5 for acidogens (Khanal, 2009). This indicates the acidogenesis was the dominated microorganism in the reactor for the first two periods. As the pH raised to 6.55 in period (3) and 6.68 in period (4), the reactor reached the optimum pH of 6.8 to 7.4 for combined culture of acidogens and methanogens ((Khanal,2009).

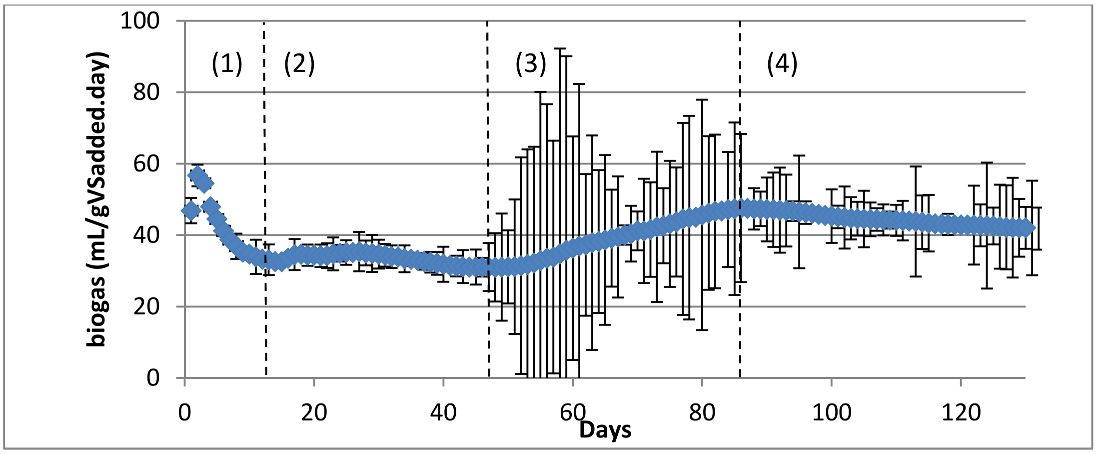


Figure 6-18: Daily biogas yield from mesophilic AD of raw DAF sludge

The highest average biogas yield of 44 mL/gVS$_{added}$, from raw DAF sludge, was produced during period (4) (i.e., day 85 to day 130). The average biogas yield during other periods was 34-38 mL/gVS$_{added}$. This result is in agreement with results reported by Woon (2012), where mesophilic AD of DAF sludge under semi-continuous conditions had an average yield of 26 mL/gVS$_{added}$.

However, the biogas yield from DAF sludge was much less than the BMP from the AD batch results from Woon et al. (2012) and Gehesha (2013), whereas the batch test of WAS and biosolids successfully predicted the biogas yield from the semi-continuous results. This can be due to the inhibition of biogas production caused by the accumulated TVFAs from daily feeding of DAF sludge. The first stage in AD is the hydrolysis of matter rich in protein and fats, which were rich in DAF sludge, by acid forming bacteria and degrading into TVFAs. If it is in a well performing AD reactor, acetoclastic methanogens further convert the TVFAs into biogas. However, the methanogens grow much slower than acidogenic bacteria (Solera et al.,2002). Due to the high initial concentration of TVFAs and the degradation of proteins and fats resulting in high TVFAs concentrations in the reactors the rate of production of TVFAs were more than the uptake rate of TVFAs by acetoclastic methanogens. As the accumulation of TVFAs also caused the pH to drop under 7, the methanogens lost optimum pH of 7.8 to 8.2 (Khanal,2009). The pH, TVFAs and TAN concentrations in the mesophilic AD reactor during the duration of the experiment are given in Table 6-7. The TVFAs in the reactor were 10,686 to 11,874 mg/L, thus higher than the TVFAs inhibition limit of 7,000 mg/L reported by Hejnfelt et al. (2009). Girault suggested that results from batch tests were able to predict the biogas yield when no inhibition occurred in the continuous conditions(Girault et al.,2012). Therefore the semi-continuous result of DAF sludge was different from the batch result due to the inhibition of TVFAs in the digester.

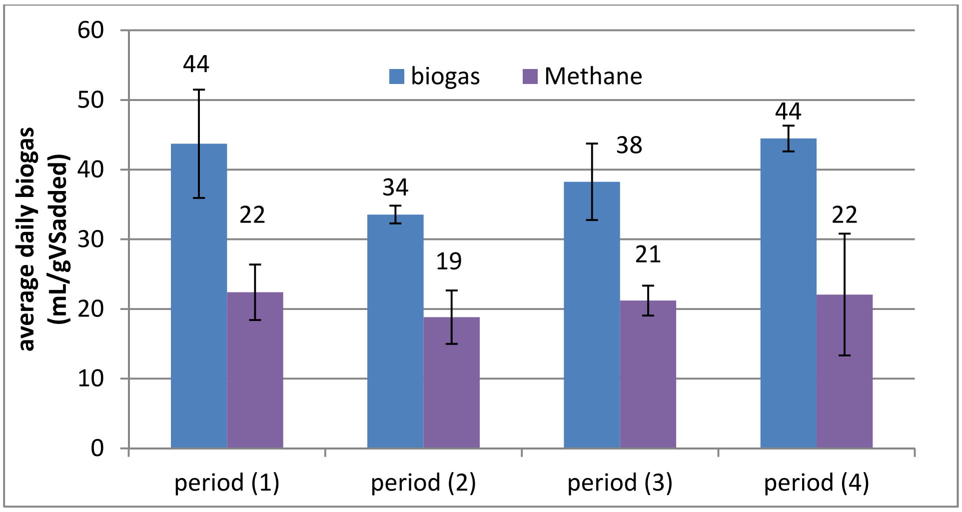


Figure 6-19: Average daily biogas and methane yield from raw DAF sludge during the experiment duration

Table 6-7: Summary of Experimental Result for mesophilic AD of DAF sludge

Period	**Day**	**Biogas**	**Methane**	**VS**	**COD**	**pH**	**TVFAs (mg/L)**	**TAN (mgN/L)**
		mL/gVS$_{added}$.day		Reduction (%)				
1	1-10	44	22	61%	40%	6.25±0.01	10,868±66.6	1604±60
2	11– 44	34	19	47%	32%	6.27±0.15	not available	2428±91
3	45- 84	38	21	22%	26%	6.56±0.26	11,874±72.8	1396±52
4	85-130	44	22	18%	35%	6.88±0.16	11,072±67.9	1240±678
Inhibition Limits						pH<7	TVFAs>7000 mg/L (Hejnfelt et al.,2009)	>1500 mgN/L (Gerardi,2003)

6.2.3.2. *AD of DAF sludge of Low TS*

As discussed in section 6.2.3.1, the AD of raw DAF sludge under continuous conditions was inefficient due to TVFAs accumulation and inhibition. Therefore, this experiment investigated the AD of DAF sludge mixed with water (i.e., lower TS and COD), in order to reduce the OLR and the TVFAs levels.

In order to operate the AD reactors at the recommended loading rate from the US EPA (1979), DAF sludge samples were mixed with tap water from slurries of 57, 31, and 14 gTS/L labelled as DAF-A, DAF-B and DAF-C respectively. Due to the variation of DAF sludge sample received over the experimental period (e.g., the TS/VS ratios), the VS of the DAF-A, DAF-B and DAF-C changed slightly over time (see Figure 6-20).

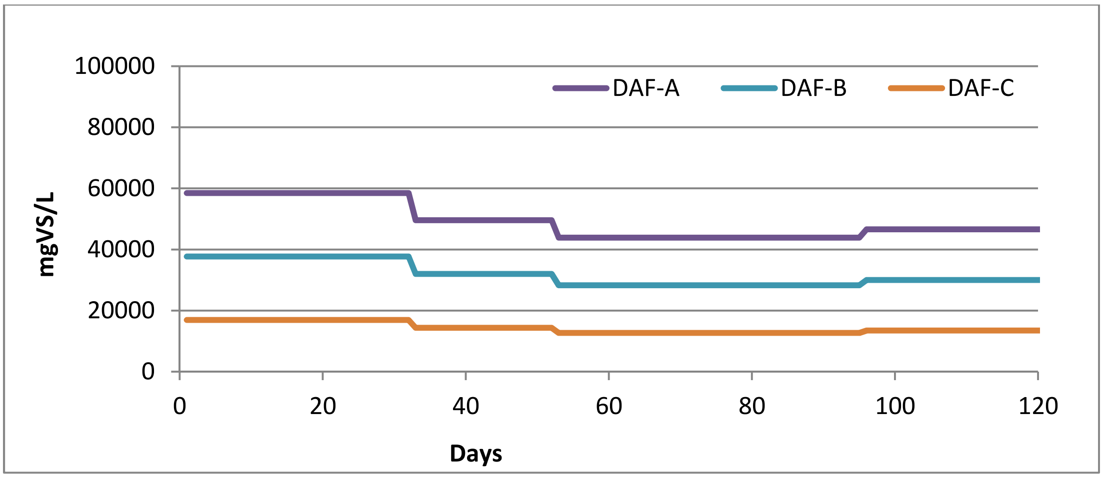


Figure 6-20: VS concentrations of DAF sludge slurries over the experiment period

Reactors fed with DAF A, DAF B and DAF C were labelled as M-14-DAF A, M-14-DAF B and M-14-DAF C for a HRT of 14 days and M-23-DAF A, M-23-DAF B and M-23-DAF C for a HRT of 23 days, respectively. The performance of the AD reactors in terms of biogas yield, effluent and biosolids quality was evaluated at a HRT of 14 and 23 days. The corresponding OLRs for each HRT and feedstock are given in Table 5-13.

On day one, DAF sludge with low TS and anaerobic raw inoculum were mixed at the ratio of 30:70 v/v (i.e., same ratios used for batch BMP tests in section 6.1). Effective from day two, the reactors were fed in accordance with the HRT.

Figure 6-21 shows the daily biogas yielded for DAF A, DAF B and DAF C feedstock. Similar to the mesophilic DAF sludge, the trend in biogas yield can be classified into four distinguished periods, period (1), (2), (3) and (4). Period (1) is the start-up period, where the biogas yield production during the first 10 days, deceased rapidly, for all DAF sludge slurries and HRT tested. This trend is in agreement with trend observed previously for mesophilic AD of WAS, biosolids and raw DAF sludge. As discussed in previous sections, the start-up up period represents the time during which biogas yield continued to decrease.

Period (2) is where biogas yield continued to decrease but at a lower rate compared to period (1). For example, the yield from M-23-C-daf decreased from 81 mL/gVS.day on day 10 compared to biogas yield of 33 mL/gVS.day on day 39.

Standard deviation in biogas production also indicates the stability of the system as the difference between the duplicated reactors, which was relatively smaller during this period compared with that in period (1). The trend in biogas yield observed during period (2) indicated reactors may undergo a regrowth of methanogenesis bacteria as the rate of the wastage gained a balance with the regrowth rate. Thus, a relative stable period was observed.

The period where biogas yield started to increase at a slower rate is designated as period (3). This increase of biogas yields indicated the acclimation of the reactors as the bacteria were getting used to the DAF sludge and becoming more tolerant of any toxic or inhibition factors. The high standard deviation of biogas yield of period (3) was caused by the difference of the duplicated reactors. Acclimation is a biological process that even with duplicated reactors showed slightly different rates of acclimation, and then caused the higher standard deviation in this period compared with the previous period. For example, the highest SD of biogas in period (2) was 12 mL/gVS compared with the highest SD of biogas yield of 30 mL/gVS in period (3) from M-23-C-daf reactor.

Period (4) had stabilised biogas production indicated by low standard deviation over the period. It was noticeable that the reactors fed with DAF C reached period (4) by day 70, whereas reactors fed with DAF A and DAF B reached period (4) by day 105 and day 110, respectively. In other words, the time of reactors reaching stable period (4) was found to be proportional to the VS concentration in the feedstock or to the OLR. Reactors fed with a higher concentration of VS were found to need a longer time to reach the stable period (4). This may be due to the high organic fraction in the reactors that caused the higher level of inhibition factors so that microorganisms took a longer time to adapt compared with reactors fed with lower VS and lower levels of inhibition factors.

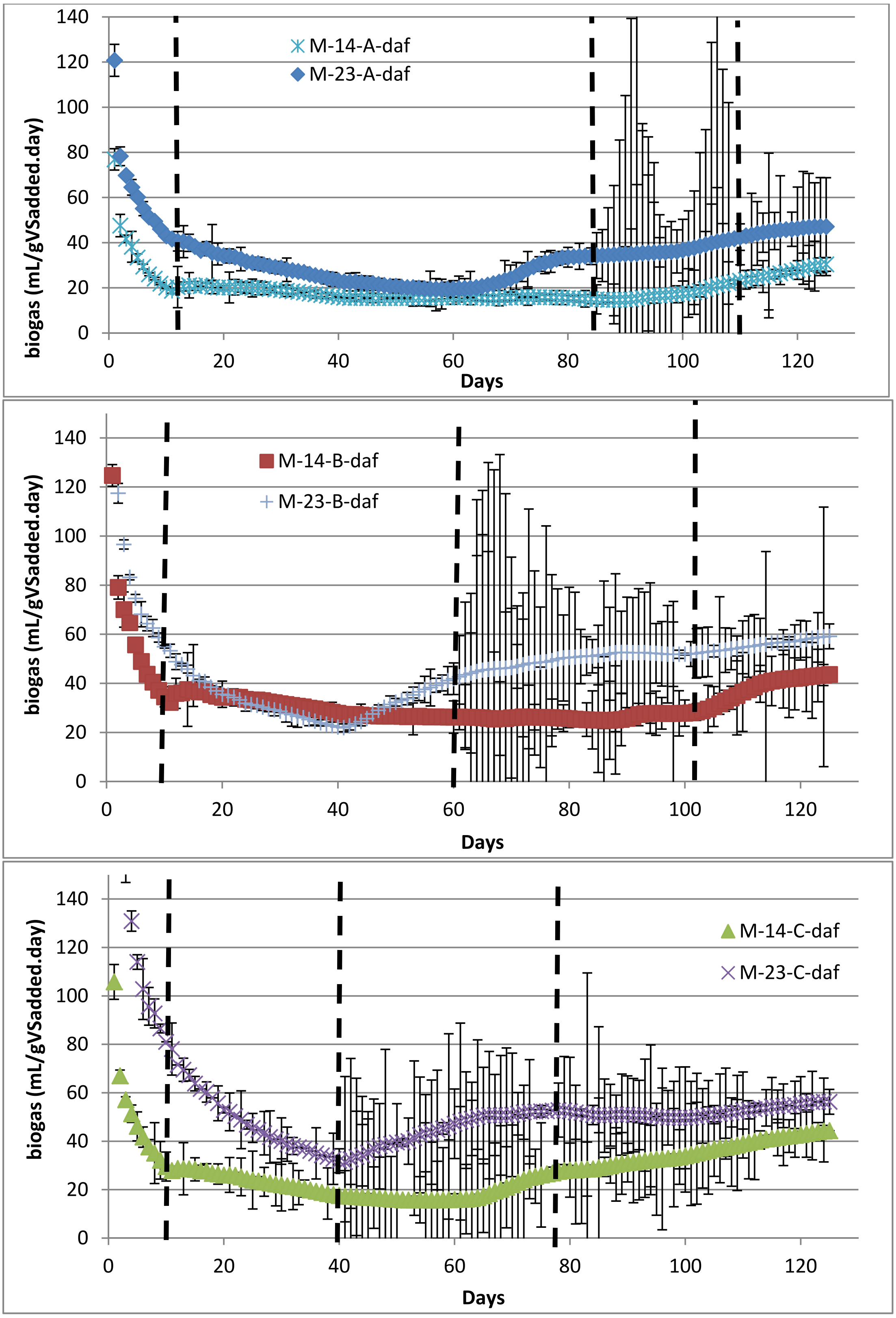


Figure 6-21: Daily biogas yield from mesophilic AD of DAF-A, DAF-B and DAF-C

The average biogas and methane yield during period (4) is shown in Figure 6-22. Overall, a higher biogas yield was obtained at the longer HRT of 23 days applied on the AD used in this experiment in comparison to shorter HRT of 14 days. For example, the biogas yield increased from 28.0 to 46.0 mL/gVS$_{added}$ with the increase in HRT from 14 to 23 days for reactors fed with DAF A. This is around a 64% enhancement in biogas production. However, for reactors fed with slurry DAF B and DAF C, their biogas yield increased by 41% for both conditions. This indicates that high OLR reactors require longer HRT to provide adequate time for the anaerobe to digest the organic matter.

Looking at the performance of AD reactors that received DAF A and DAF B, it was observed that higher biogas production was obtained in reactors receiving lower strengths of the DAF sludge slurry. For example, the yield at a HRT of 23 days from reactors with DAF A and DAF B was 33 mL/gVS.day and 43 mL/gVS.day, respectively, where the strength of DAF sludge was higher in DAF A than DAF B (see Figure 6-22). However, the use of lower OL (i.e., DAF C) showed a negative effect where the biogas at a HRT of 23 was 53 mL/gVS.day (i.e., around 7% lower biogas yield compared to that using DAF B). This indicates the reactors that received DAF sludge C were under-loaded. In other words, the AD reactors that received DAF sludge slurry B operated at the optimum OLR. Overall the highest biogas yield of 56.9 mL/gVS$_{added}$ obtained for DAF B at a HRT of 23 days was 28% higher than the biogas yield from mesophilic AD of raw DAF sludge.

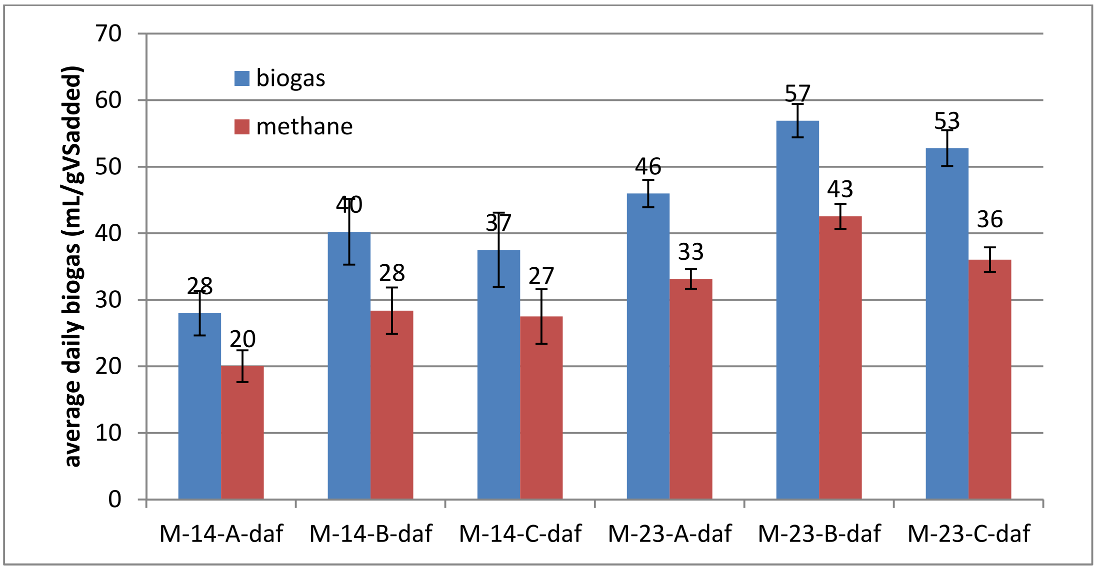


Figure 6-22: Average daily biogas and methane yield of diluted DAF over period four under mesophilic AD

The contour plot in Figure 6-23 shows higher HRT produced higher biogas yield within tested conditions. In the longer HRT range, OLR had less significant effects on biogas yield compared with short HRT. The optimum OLRs were 1.1 to 1.6 g VS/L.day under a HRT of 23 days.

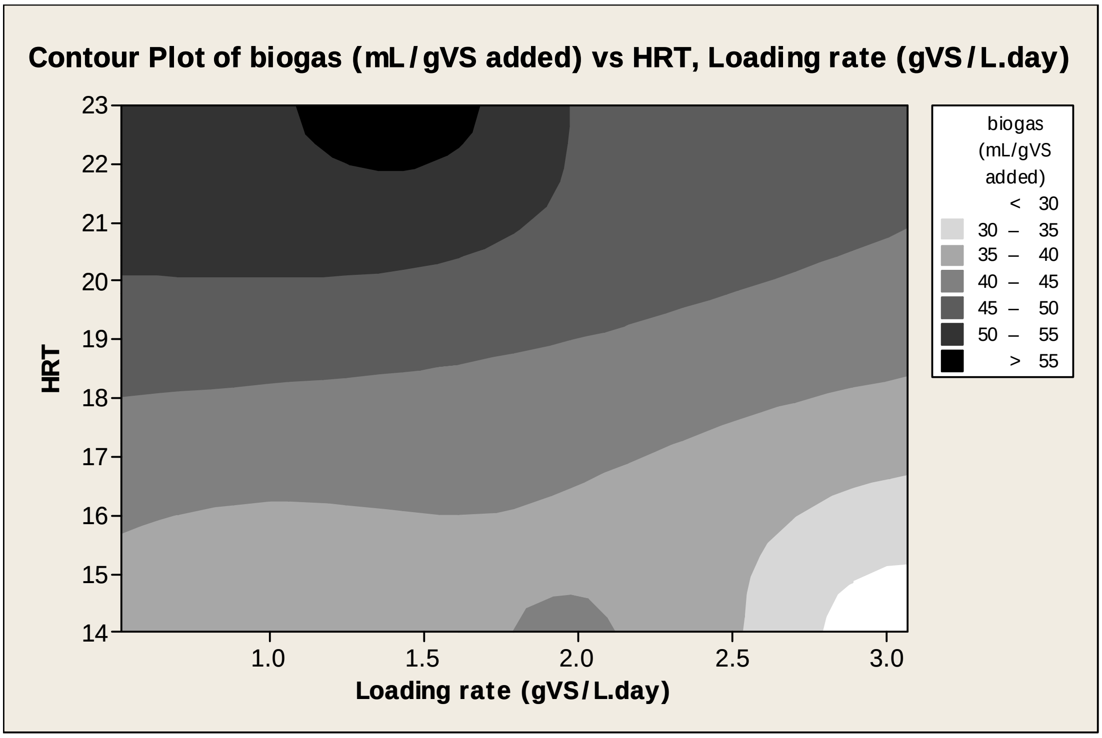


Figure 6-23: Biogas yield versus OLR and HRT for mesophilic AD of diluted DAF

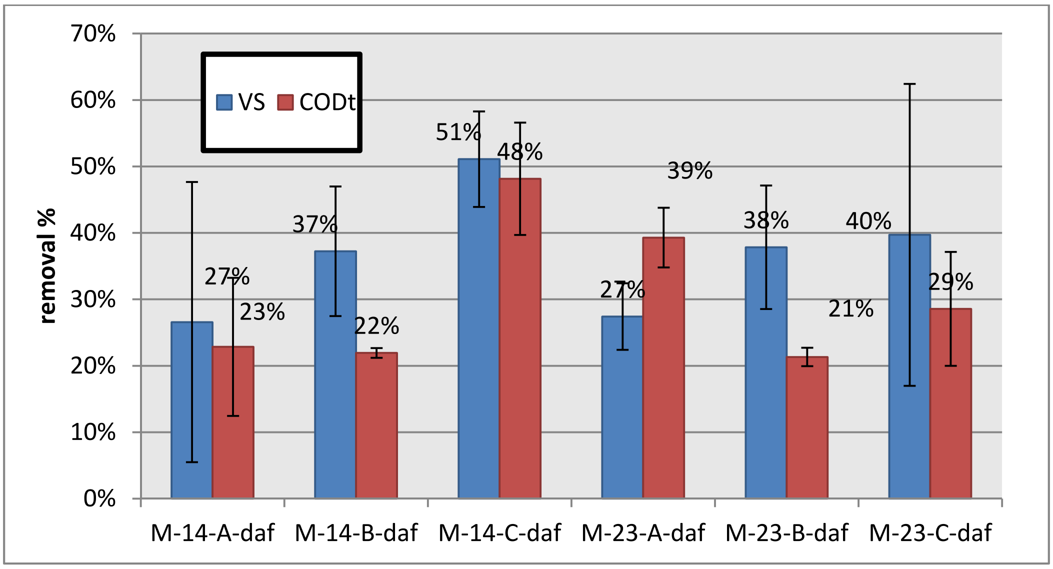


Figure 6-24: CODt and VS Removal using mesophilic AD of DAF sludge low TS (slurry A, B and C) for period four.

In terms of the organics removal, the average VS reduction was inversely proportional to the OLR. For example, VS reduction of 27%, 37% and 51% was obtained with decreased OLRs of 3.9 gVS/L, 2.6 gVS/L and 1.2 gVS/L(i.e., DAF A, DAF B and DAF C), respectively, at a HRT of 14 days. The same trend was observed at a HRT of 23 days. This is higher than the VS reduction of 7% in mesophilic AD of raw DAF during stabilised biogas production, period (4). The average CODt removal increased from 22% to 48% in with the decrease in OLR from 2.6 gVS/Lto 1.2 gVS/Lfor mesophilic AD of DAF sludge with low TS at a HRT of 14 days.

When considering the effluent quality, both TVFAs and ammonia in effluent from reactors were tested. Since the DAF sludge used in this experimental run had low TS to reduce the OLR and TVFAs levels of the feed, the results showed the TVFAs and ammonia in the digester were all lower than the reported inhibition level of 7,000mg/L for TVFAs and 1,500 mg/L for ammonia. Another reason to test the effluent is because of the trade waste limits; any parameter that exceeds the criteria will comprise an extra cost for the industries. In this test, effluent from reactors fed with DAF A and DAF B exceeded the trade waste limits for TVFAs (organic acid) of 1,000mg/L and for ammonia of 200 mg/L (see Table 6-8). Effluent from reactors fed with DAF C met the trade waste criteria for TVFAs, but not for ammonia. Since ammonia is the by-products from the AD process, it is usually higher in the effluent than the influent

Table 6-8: Effluent Quality of Mesophilic AD of DAF sludge with Low TS

Reactors	pH	TVFAs (mg acetic acid/L)	TAN (mgN/L)
Trade waste limits (CWW)	6.00-10.00	1000	200
M-14-DAF-A	6.89±0.18	2971±170	957±170
M-14-DAF-B	6.84±0.26	1599.3±141	599±141
M-14-DAF-C	6.92±0.17	751±26	289±26
M-23-DAF-A	6.93±0.18	3453±104	1280±104
M-23-DAF-B	6.91±0.28	1323±67	741±67
M-23-DAF-C	7.12±0.18	407±49	267±49

Those effluents were also tested for their settling properties by allowing them to settle by gravity for four hours. A layer of floating scum was found in most of the reactors, except the M-23-C-daf. With higher organic content in the feed, the scum layer was thicker. The floating sludge indicates dispersed particles or fine particles and that the gravity steeling is not adequate. The layer may also be caused by the lipids in the DAF sludge, which has a tendency to form floating scum(Salminen, E et al.,2002a). Polymer can be used to allow coagulation of particles for the dewatering process.

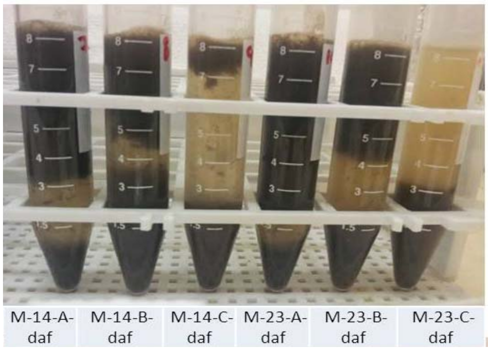


Figure 6-25: Settling conditions of diluted DAF after mesophilic AD

Table 6-9: Summary of mesophilic AD of diluted DAF

Reactors	HRT day	OLRs		Biogas	Methane	COD Removal	VS Removal
		gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day			
M-14-DAF-A	14	0.24	3.07	28	20	23%	27%
M-14-DAF-B	14	0.26	1.98	40	28	22%	37%
M-14-DAF-C	14	0.28	1.00	37	27	48%	51%
M-23-DAF-A	23	0.17	2.46	46	33	39%	27%
M-23-DAF-B	23	0.19	1.40	57	43	21%	38%
M-23-DAF-C	23	0.17	0.52	53	36	29%	40%

6.2.3.3. *AD of Non-polymer DAF sludge*

In the operation of DAF sludge units, a polymer is added to enhance the thickening of the sludge and hence improve the performance of the DAF unit. Polymer has been reported for significant reduction of anaerobic biodegradability with polymers at acetylation levels of between 1.2 to 1.7 (Rivard et al.,1992).

This section discusses the effect of the polymer on the AD of DAF sludge. The feedstock used in this experiment was prepared from the influent to the DAF-unit, (i.e., the pre-DAF stream before the polymer is added) using a centrifuge to thicken it.

The results obtained for DAF sludge of low TS showed that the AD of DAF sludge of low TS at a HRT of 23 days had better biogas yield, VS and COD removal. Hence, the non-polymer DAF sludge was mixed with water to reduce the TS and COD to the designated level (i.e., 13,000 to 16,000 mg/L. The mesophilic reactors receiving non-polymer DAF sludge were operated at OLRs of 0.62 g VS/L.day and HRT of 23 days, labelled as NM23.

Unlike the start-up of mesophilic of diluted AD (see section 6.2.3.2), the experiment used similar approach as batch tests where the inoculum and substrate were mixed at the ratio of 70:30v/v on day one. AD of the non-polymer DAF sludge started with the substrate to inoculum ratio according to HRT on day one onwards. This is because section 6.2.3.2 showed possible initial overloading, which caused the slow biogas yield during first and second period over the four periods. Since the initial starting condition was different from mesophilic AD of DAF sludge of low TS, in order to compare with AD of DAF sludge of low TS, another set of mesophilic AD of DAF sludge of low TS reactors ran at the same time with NM23 using the substrate to inoculum ratio according to HRT on day one onwards. The DAF sludge of low TS had similar VS content and under the HRT of 23 and 30 days, which were labelled as HM23 and HM30.

Figure 6-26 shows the daily biogas yield from mesophilic AD of non-polymer DAF sludge (NM23) and non-polymer DAF sludge of low TS (HM23 and HM30). The daily biogas yield from the reactor NM23 increased during the first 18 days, then continued to gradually decrease until day 80. Afterward, biogas yield stabilised at about 76 mL/gVS.m^3 (see Figure 6-27). The reactors HM23 and HM30 showed stabilised production after 20 and 80 days, respectively.

It was observed that biogas production from all three reactors, NM23, HM23 and HM30, increased during the start-up period. This is opposite to the trend observed in section 6.2.3.2 AD of DAF sludge with low TS (see Figure 6-21). The main difference between the two set of reactors was the OL applied on day one. The latter received substrate to inoculum at a ratio of 30:70 v/v, but according to HRT from day two onward, whereas the former received OL based on the designated hydraulic retention time.

Though running at the same HRT, NM23 and HM23 showed quite different behaviour in terms of the time reaching the stable status (see Figure 6-26). Instead, the HM30 reactor displayed more alike trends, with NM23 as daily biogas yield shifting to the stable end.

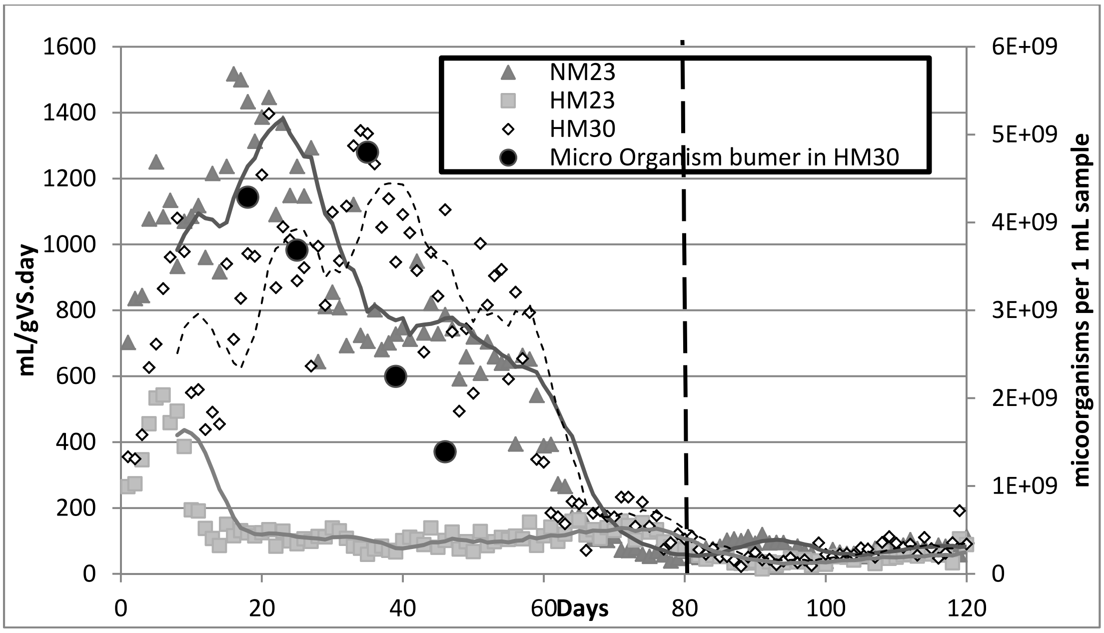


Figure 6-26: Daily biogas yield from mesophilic AD of non-polymer DAF and diluted DAF started with the substrate to inoculum ratio according to HRT on day one onwards

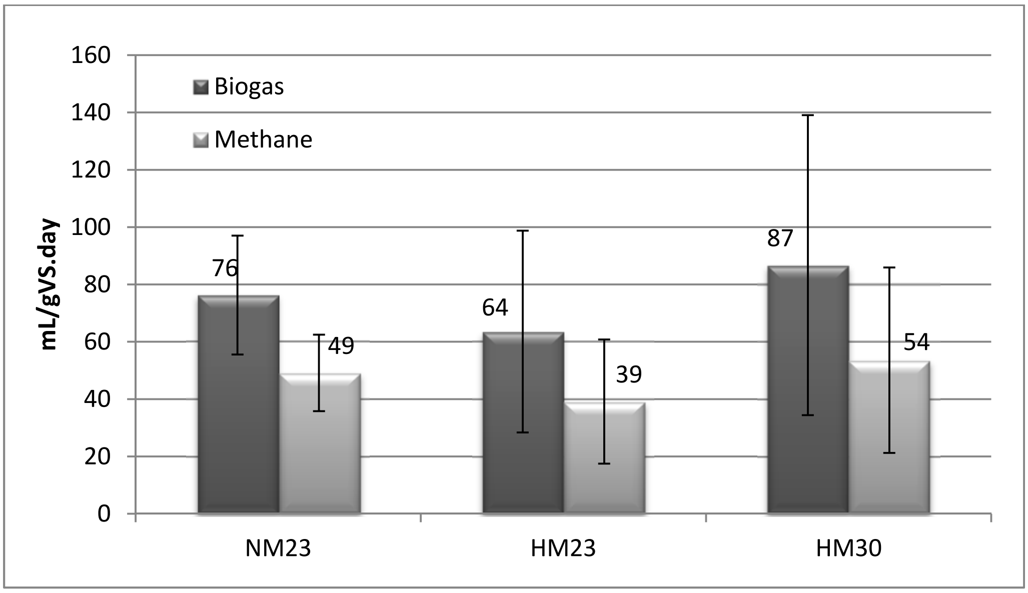


Figure 6-27: Average biogas and methane yields from non-polymer DAF sludge and DAF sludge of low TS under mesophilic AD (over the stable period)

As shown in Figure 6-27 non-polymer DAF sludge (NM23, OL = 0.62 g VS/L.day) showed 20% higher biogas yield and 23% higher methane yield. These results indicate that the addition of polymer to the DAF sludge has a negative effect on its degradation under mesophilic AD. This could be due to the hydrophobic nature of the polymer, which reduces wetting and thus enzyme/microbe surface contact (Rivard et al.,1992). The other factor, is polymers bond the sludge into floc, hence the organic compounds become trapped in these floc. This limits the anaerobic microorganism from access to their food source. Another hypothesis is the steric interference presented by polymer may reduce the enzymatic activity and thus slow digestion of organics (Rivard et al.,1992) .

The reactor that received non-polymer DAF sludge (NM23) had higher VS reduction compared to DAF sludge (HM23), both of which operated at HRT of 23 days. Further, non-plymer DAF sludge showed higher VS reduction that the DAF sludge treated at longer HRT of 30 days (see Figure 6-28). Under same HRT and similar OLR, non-polymer DAF sludge showed 57% higher VS reduction compared with industry DAF sludge in HM23, that corresponds with the improved biogas yields from non-polymer DAF sludge.

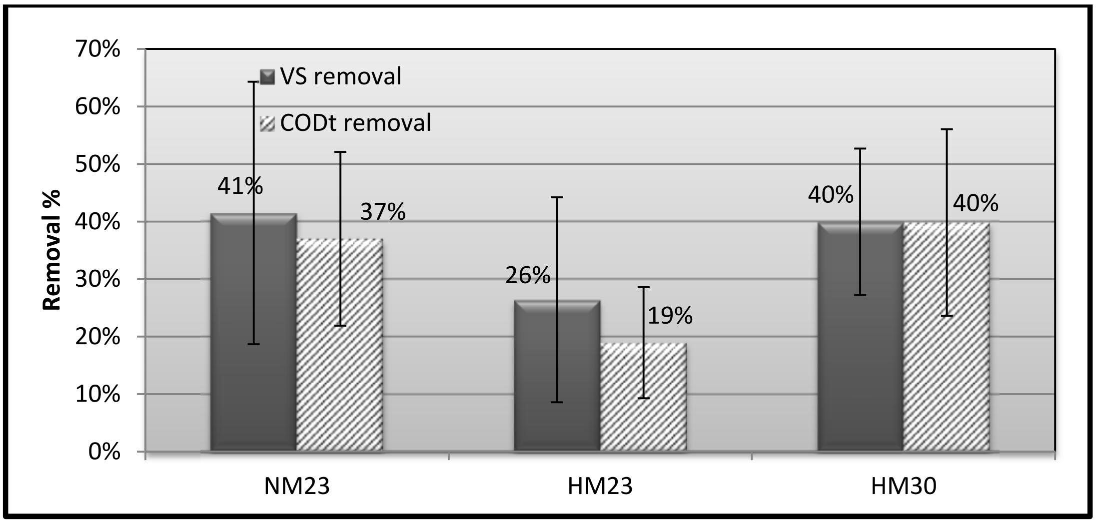


Figure 6-28: Organic removal in the mesophilic AD of non-polymer DAF sludge and DAF sludge of low TS

When considering the effluent quality, the CODs, TVFAs and ammonia of effluent from all reactors exceeded the trade waste limit, but less than the inhibition limits reported by the literature. Table 6-10 lists some trade waste parameters. Though the CODs, TVFAs and TAN from NM23 exceeded the trade waste limits, they were less than effluent than the AD of diluted DAF sludge by 18% (HM23).

Table 6-10: Effluent quality of mesophilic AD of non-polymer DAF sludge and DAF sludge of low TS (during the stabilised period)

Reactors	pH	CODs (mg/L)	TVFAs (mg/L)	TAN (mgN/L)
NM23	6.98±0.05	1935±1354	1191±203	315±76
HM23	7.09±0.10	2907±2059	1676±566	468±142
HM30	7.18±0.09	2543±1840	1457±75	329±114
Trade Waste Limits (CWW)	6-8	1000	1000	200

Table 6-11: Summary of mesophilic AD of non-polymer DAF sludge

Reactors	HRT day	OLRs		Biogas	Methane	COD Removal	VS Removal
		gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day			
NM23	23	0.24	0.62	76	49	37%	41%
HM23	23	0.17	0.65	64	39	19%	26%
HM30	30	0.16	0.50	87	54	40%	40%

6.2.3.4. *AD of DAF sludge of Low TS Using Acclimated Inoculum*

There are a limited numbers of anaerobic digesters being operated on for the treatment of wastes from the meat industry. Therefore, a 5 L mesophilic AD reactor was used to acclimatise inoculum to DAF sludge. The previous sections discussed AD of raw DAF sludge and DAF sludge slurries using inoculum from a wastewater treatment plant. Therefore the aim of this experiment was to assess the effect of the use of inoculum acclimatised to the DAF sludge on DAF sludge biogas yield. .

The results discussed in sections 6.2.3.1 to 6.2.3.3 showed that AD of DAF sludge has lower biogas yield compared to WAS (e.g., biogas yield for DAF sludge 64mL/gVS.d compared to 188mL/gVS.day for WAS), and VS reduction of 26% for DAF sludge at OLR of 0.65 g VS/L.day compared to 18% for WAS. Therefore, this experiment aims to investigate the effect of inoculum on biogas yield.

The use of acclimatised inoculum to carry out BMP experiments has been reported to have better tolerance to high ammonia and TVFAs, as indicated through better performance (Abouelenien et al.,2009; Güngör-Demirci et al.,2004; Woon et al.,2012a). Section 0 showed the initial ratio of inoculum has great effect on the stabilisation of daily biogas yield. Hence, this test aimed to explore the effect of initial inoculum on AD reactors.

DAF sludge of low TS was used for feeding the reactors where VS content of the feed was 17 gVS/L, similar with the DAF C in section 6.2.3.2. Reactors started with the substrate to acclimated inoculum ratio according to HRT on day one and onwards ran under a HRT of 23 and 30 days, and were labelled as HM23-acc and HM30-acc, respectively. Reactors started with the substrate to raw inoculum (non-acclimated inoculum) ratio according to HRT on day one and onwards ran under a HRT of 23 and 30 days, and are labelled as HM23-non and HM30-non, respectively. This is regarded as baseline results since they were run at the same time with the same feeding stock, and confirmed the pervious findings in section 6.2.3.2.

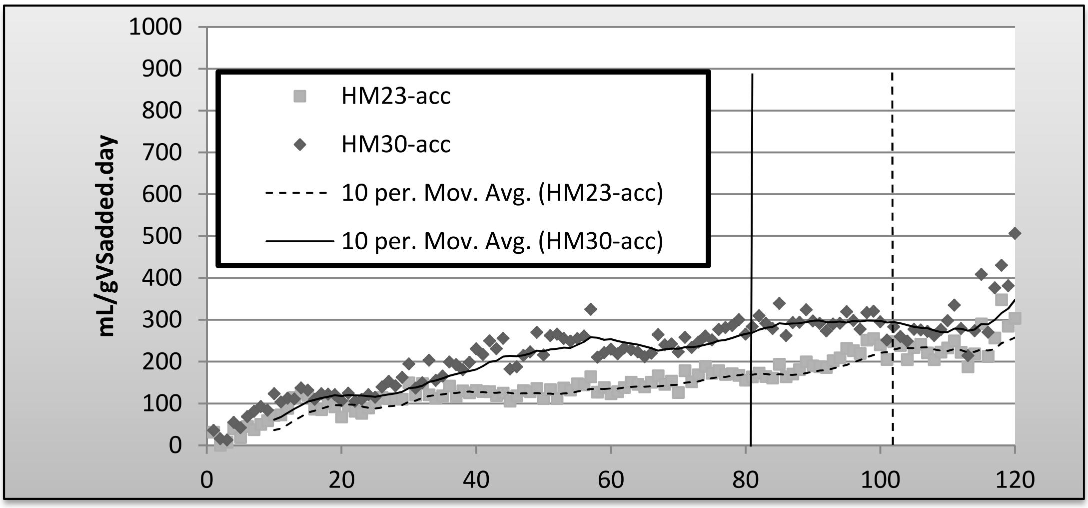
Figure 6-29 shows the daily biogas of DAF sludge of low TS with acclimated inoculum and raw inoculum. This differs from the literature stating acclimated sludge improved AD performance. Our results showed the raw inoculum (non-acclimatised) had much higher biogas yield over the first three cycles of HRT. However, the raw inoculum showed significant instability since there was much greater variation of daily biogas yield. Further, after three cycles of HRT, the reactors with raw inoculum reduced the biogas yield dramatically, for instance, the daily biogas production from reactor HM23-non dropped from 612 mL/gVS$_{added}$ on day 63 to 155 mL/gVS$_{added}$ on day 85. The biogas yield then became more stable after the sudden drop down. HM30-non reached the stable period around day 115.

A similar phenomenon was observed in Figure 6-26, where the biogas yield decreased significantly over time. However, though DAF sludge of low TS ran at the same retention time, same start-up inoculum ratio and similar OLR, HM30 became stable on day 80 in Figure 6-26, whereas HM30-non reached the stable period on day 110 in Figure 6-29. The two experiments were carried out at different times, though samples were from the same facility, and the VS of raw DAF sludge used in section 6.2.3.2 was 140 gVS/L and was 58% higher than the VS content in raw DAF sludge used in this experiment. Hence, the variation of DAF sludge sample due to the different production lines in operation increased the level of complexity of the studies.

Reactors with acclimated inoculum had much more stable gas yields after one cycle of HRT and were followed by a gradual increase of gas yield until being stable around day 80 for

HM23-acc and around day 102 for HM30-acc. As mentioned, the acclimated inoculum was taken from a laboratory reactor fed with DAF wastewater, which had much lower OLR compared with DAF sludge. Hence, the gradual increase of biogas yield from reactors with acclimated inoculum is likely caused by the increasing OL feeding into the reactors at the start-up. Since the acclimated inoculum had adopted the DAF sludge as its feeding source, with increasing loading, the bacterial were also grown without being inhibited or more tolerant of the inhibitors. Thus, reactors reached stability faster and yielded higher biogas than reactors started with raw inoculum.

The average biogas and methane yield after reactors reached stable status is shown in Figure 6-30. Similar to the AD of the other substrate, the biogas yield increased from 228 mL/gVS$_{added}$ to 282 mL/gVS$_{added}$ when the HRT increased from 23 to 30 days in acclimated inoculum reactors. Also, the acclimated inoculum produced higher biogas if not counting the unstable period; for example, at the same HRT of 23 days, reactor HM23-acc produced 6% higher biogas compared with reactor HM23-non.



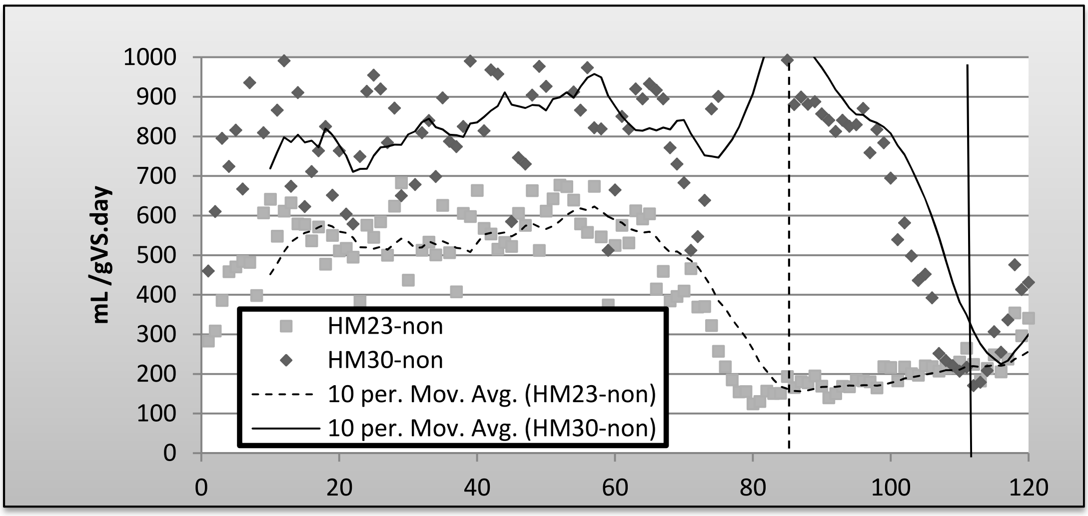


Figure 6-29: Average daily biogas production from DAF sludge of low TS with acclimated inoculum and raw inoculum (vertical line indicates the start of stable period)

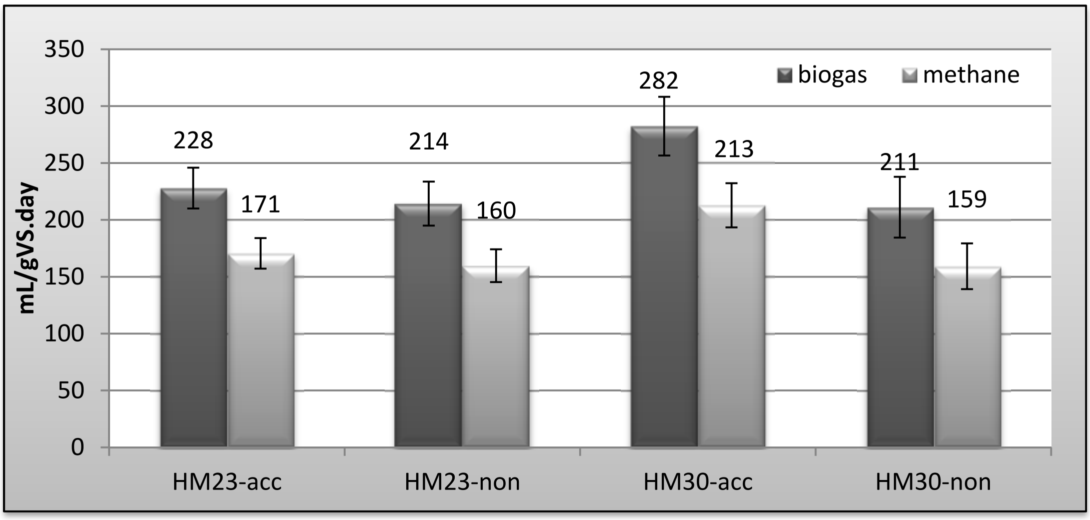


Figure 6-30: Average biogas and methane yields from diluted DAF sludge with acclimated inoculum

The average VS reduction in mesophilic AD of DAF sludge of low TS ranged from 53% to 56% at various conditions (see Figure 6-31). However, with the extended HRT, unlike the biogas yield, the VS reduction was not improved. The same trends applied to the total COD. The average CODt reduced 51 to 58% at acclimated reactors and 60 to 63% under raw inoculum reactors. The reduction was higher than the AD of DAF sludge of low TS started with a substrate to inoculum ratio of 30:70v/v on day one and according to HRT onwards, which had CODt reduction, and ranged 22 to 48%. Overall, the acclimated inoculum did not provide further reduction of VS and CODt in the healthy reactors.

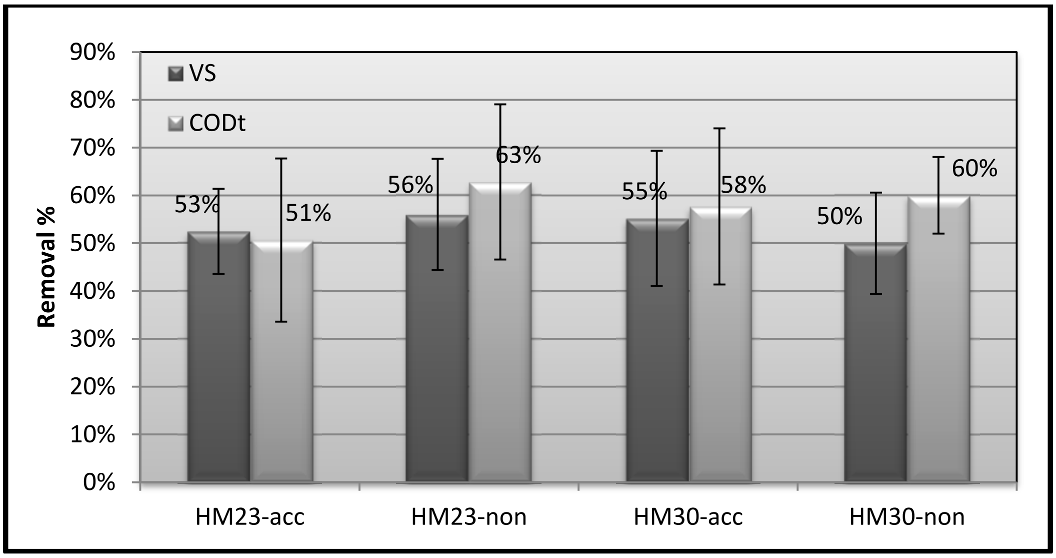


Figure 6-31: Organic removal rate in the diluted DAF AD reactors with acclimated inoculum

The concentration of TVFAs, pH, CODs and TAN in the effluent from reactors HM23-acc, HM30-acc, HM23-non and HM30-non were tested. It was observed that TAN was the only parameter that exceeded the trade waste limit of 200 mg/L. On average, ammonia excessed the limit by 91 to 224 mg/L. It was also noted that the reactors that received acclimatised inoculum had 13% less TAN in the effluents under both HRT compared with reactors started up with raw inoculum.

Effluents from the reactors were allowed to settle by gravity for 24 hours. As previous tests showed floating sludge from mesophilic AD of diluted DAF after four hours, the settle time was increased to compare if longer settling time improved performance. Figure 6-33 showed the reactors with acclimated inoculum had floating layers after 24 hours settling whereas the reactors with raw inoculum improved settling after 24 hours and had no floating sludge layers.

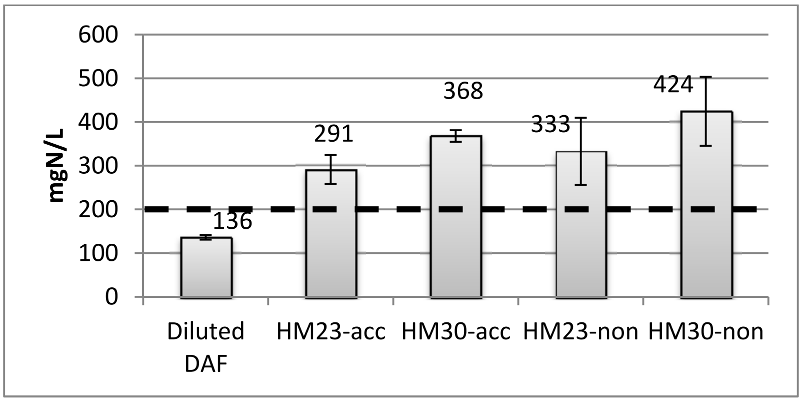


Figure 6-32: TAN in the supernatant of DAF sludge of low TS AD

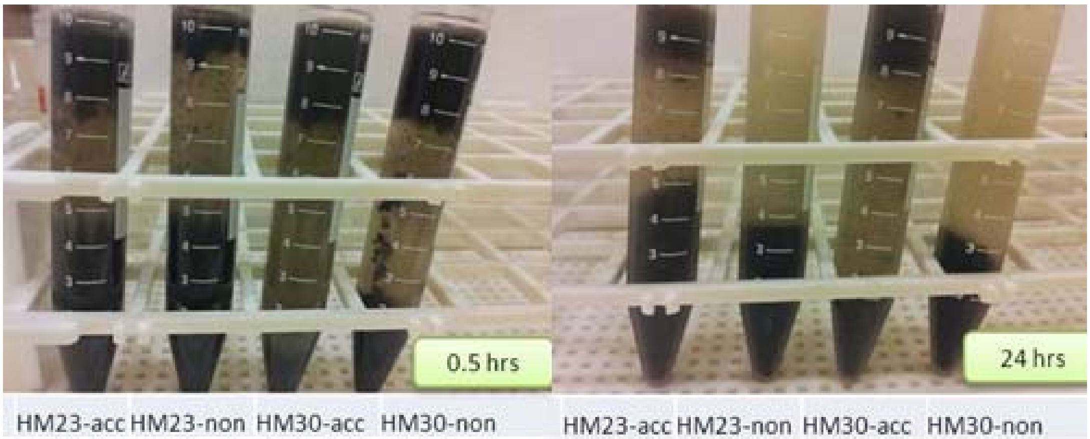


Figure 6-33: Settling conditions of DAF sludge of low TS with acclimated inoculum after mesophilic AD

Table 6-12: Summary of Mesophilic AD of DAF Sludge of Low TS with Acclimated Inoculum

Reactors	HRT day	OLRs		Biogas	Methane	COD Removal	VS Removal
		gCOD/gVS	g VS/L.day	mL/gVS_{added}.day			
HM23-acc	23	0.21	0.58	228	171	51%	53%
HM23-non	23	0.14	0.58	214	160	63%	56%
HM30-acc	30	0.19	0.44	282	213	58%	55%
HM30-non	30	0.16	0.44	211	159	60%	50%

6.2.4. **Mesophilic Codigestion of WAS and DAF Sludge Under Semi-continuous Condition**

Previous work showed that DAF sludge slurries of low TS had better effluent quality and biogas yield compared with AD of raw DAF sludge. DAF sludge slurries were prepared using tap water. Another alternative for preparing DAF sludge slurries of low TS was mixing DAF sludge with WAS from a domestic wastewater treatment plant, being of a low TS of around 2%. The wastewater treatment plant selected was looking into alternative management of their waste. According to the literature, codigestion of WAS with other wastes enhances biogas production (Long et al.,2012; Marañón et al.,2012; Wan et al.,2011). Therefore, the aim of this experiment was to assess the feasibility of codigestion of DAF sludge and WAS.

In order to compare the results with the mesophilic AD of DAF sludge of low TS, this experiment was designed using similar conditions. As mentioned in section 6.2.3.2, WAS and DAF sludge were mixed to form the similar VS content of DAF A, DAF B and DAF C, which were then named as slurry A, slurry B and slurry C, respectively (see Figure 6-34). Reactors fed with slurry A ran under a HRT of 14 and 23 days and were labelled as M-14-A and M-23-A, respectively. Reactors fed with slurry B ran under a HRT of 14 and 23 days and were labelled as M-14-B and M-23-B correspondingly. Reactors fed with slurry C ran under a HRT of 14 and 23 days and were labelled as M-14-C and M-23-C, respectively.

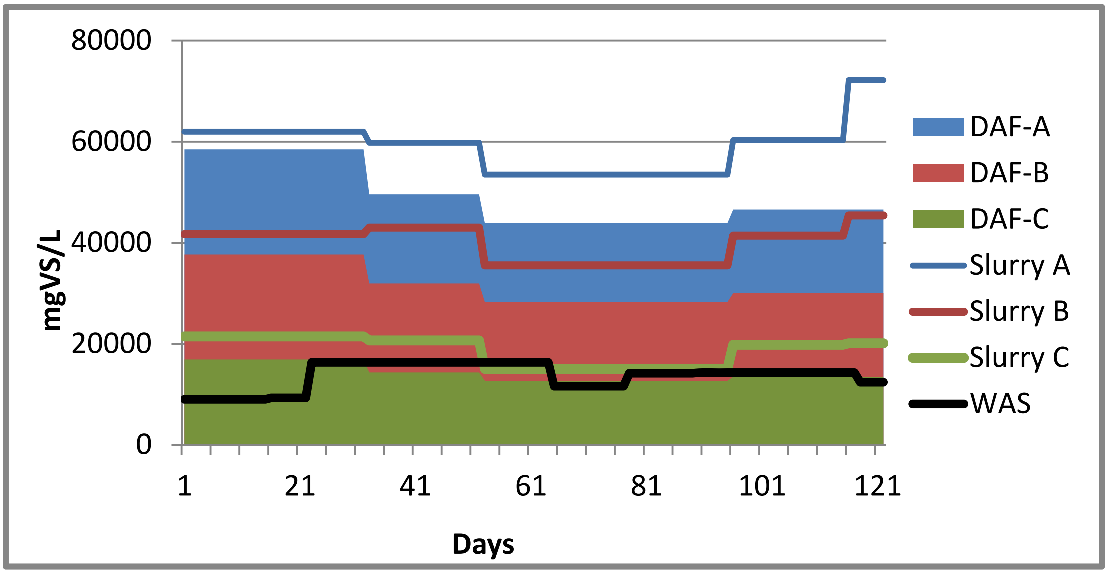


Figure 6-34: VS concentrations of DAF sludge of low TS, WAS and DAF sludge and thickened WAS

Though section 6.2.3.2 found that the reactors started with a substrate to inoculum ratio of 30:70 v/v on day one may lead to an overloaded and stressed environment, this codigestion of WAS and DAF sludge experiment was carried out at the same period with mesophilic AD of DAF sludge of low TS (see section 6.2.3.2) and used the same start-up approach with the mesophilic AD of DAF sludge of low TS. The idea was the DAF sludge would be diluted and its loading would be much lower than raw DAF sludge, so that having more substrate in the reactor in the beginning could shorten the time to reach a stable period, but not overload the reactor. However, the results from section 6.2.3.2 and this experiment showed the reactors were under stress nevertheless, although DAF sludge was diluted to a much lower VS content.

Four distinct periods were observed, similar to mesophilic AD of DAF sludge of low TS (see Figure 6-22 and Figure 6-21). Period (4), which is the stable period, started around day 100 for reactors fed with slurry A, around day 95 for reactors fed with slurry B and around day 70 for reactors fed with slurry C. The results confirmed the relationship between the organic content in the feed and the time reactor reaching stability, which was also found in mesophilic AD of DAF sludge of low TS. The lower the organic content in the feed, the shorter time required to stabilise the reactor. As explained before, this is mainly due to the overloading in the digester recovering over time. For higher organic content in the feed, reactors received more stress at the start and took a longer time to balance the reactors.

The average daily biogas and methane yielded from mesophilic AD of DAF sludge of low TS F, WAS and codigestion of WAS and DAF sludge are shown in Figure 6-36. The highest biogas yield of 188 mL/gVS$_{added}$ was found at mesophilic AD of thickened WAS under a HRT of 23 days. A Minitab main effects plot is used to analyse the effect on biogas yield when considering the feed materials, HRT and OLRs as variables.

Figure 6-37 indicates that the HRT of 23 days had better biogas yield compared with HRT of 14 days. In terms of waste materials, mesophilic of WAS yielded much better biogas compared with other material or combination tested. However, when there was codigestion of WAS and DAF sludge, biogas yield enhanced compared with mesophilic DAF sludge of low TS. For instance, biogas yield from M-23-C was 39% higher than M-23-C-daf, but it was 118% lower than WM23.

There is much research in the literature that reported biogas enhancement when there was codigestion of different waste; however, one paper found a detriment of methane yield by 33% with the addition of fat oil and grease (FOG) at 1.8% v/v to WAS at the HRT of 30 days, and 50% of methane loss with the addition of 0.2% v/v at the HRT of 26 days (Martínez et al.,2012).

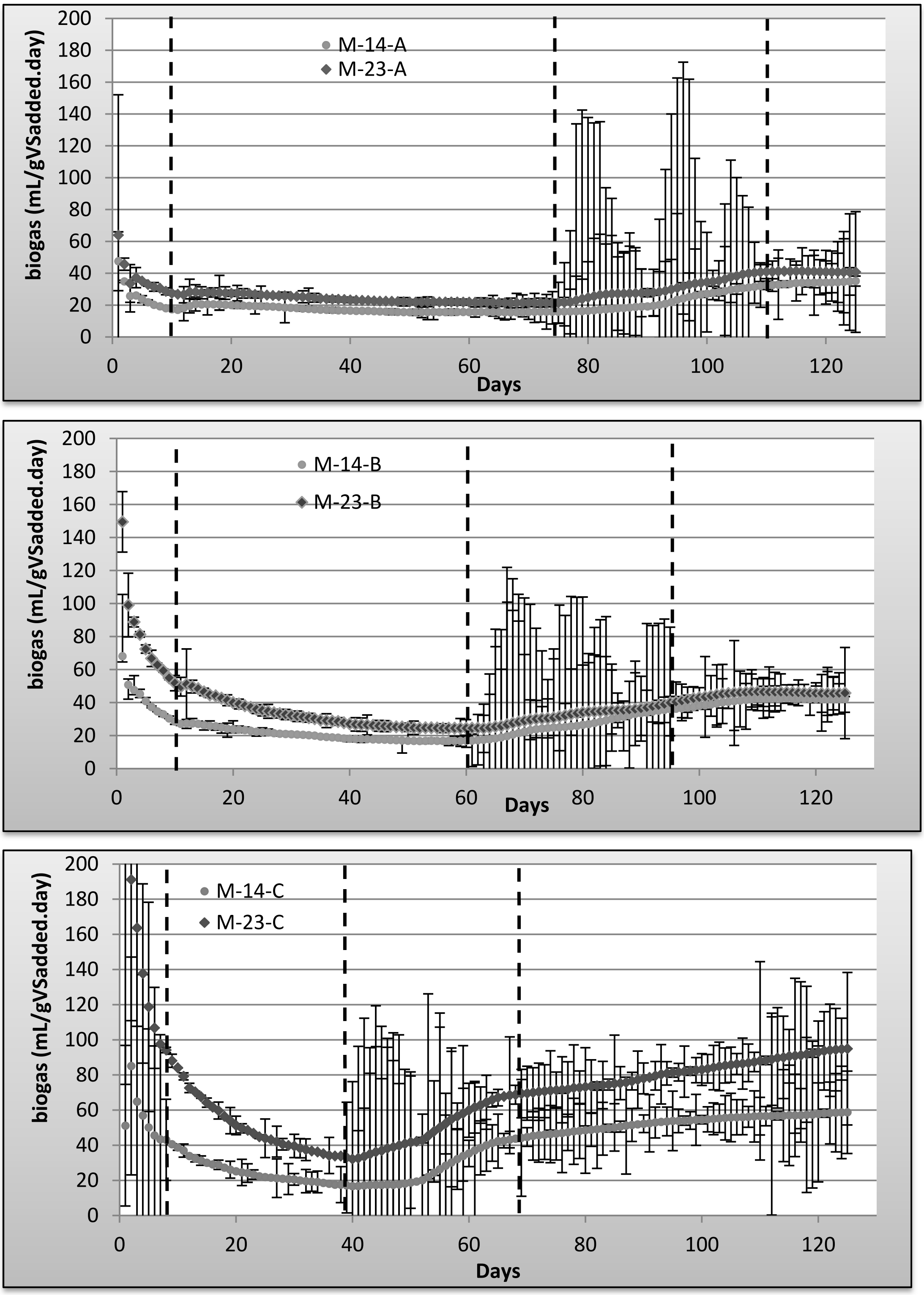


Figure 6-35: Daily biogas yield from mesophilic AD of WAS and DAF sludge

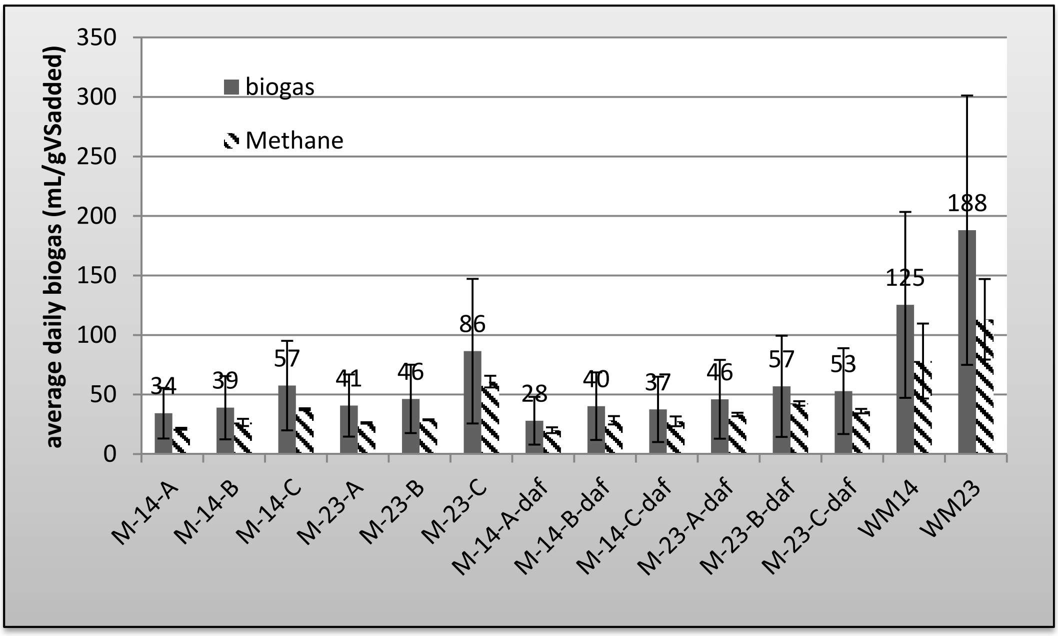


Figure 6-36: Average biogas and methane from mesophilic AD of DAF sludge of low TS, thickened WAS and WAS and DAF sludge in period (4)

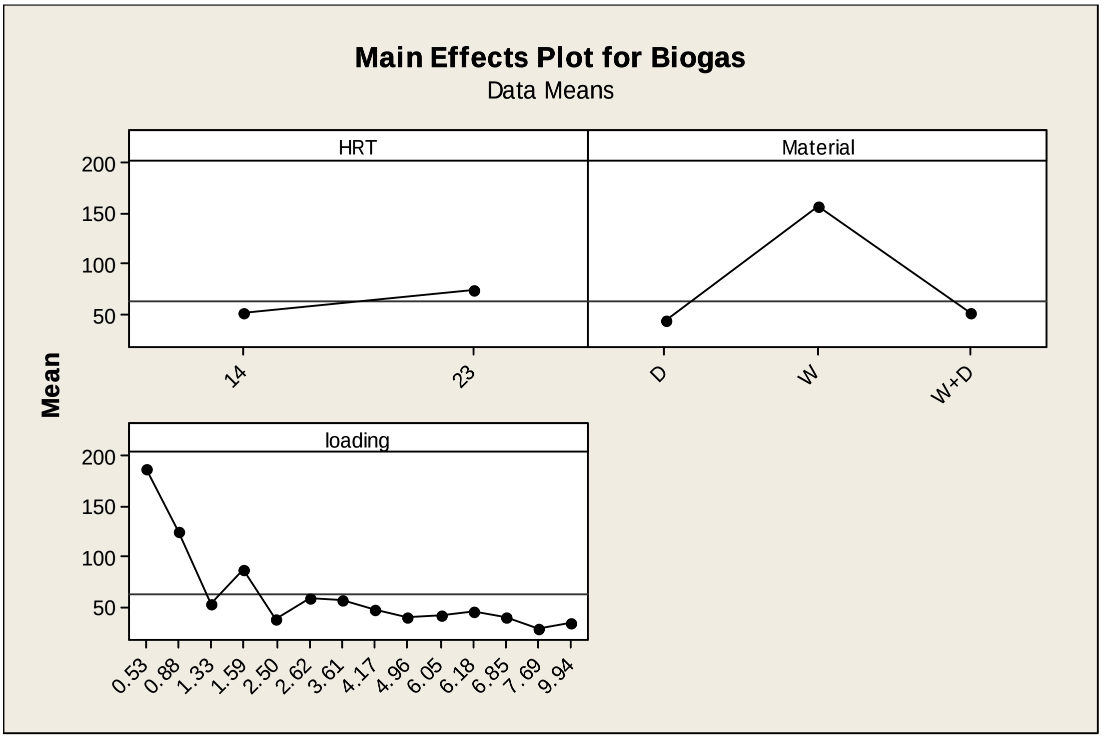


Figure 6-37: Main effects plot of biogas yield from mesophilic AD of diluted DAF sludge (D), WAS (W) and WAS and DAF sludge (W+D)

The average VS reduction for WAS and DAF was obtained using mesophilic AD ranging from 16% to 34% at various conditions (see Figure 6-38). However, with the extended HRT, unlike the biogas yield, the VS reduction was not improved. The average CODt reduced 20 to 30%, which is similar with the AD of DAF sludge during period (3) and (40, which had CODt reduction ranging from 8 to 35%.

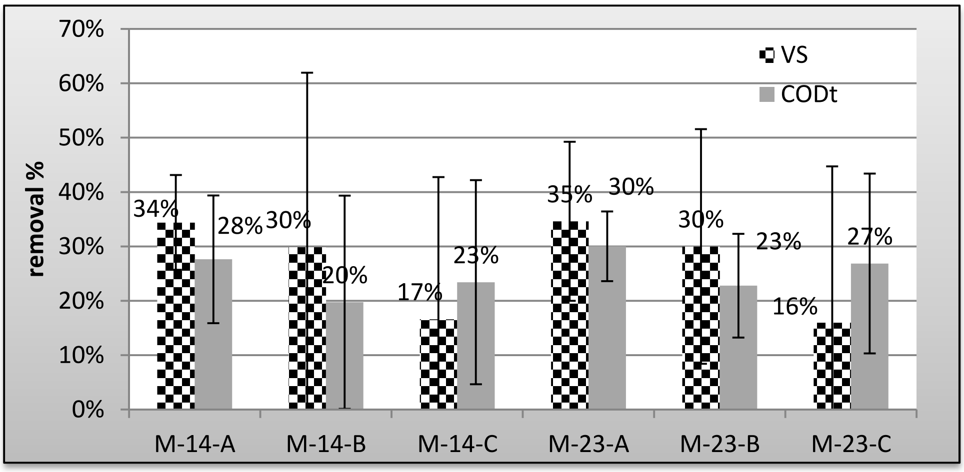


Figure 6-38: Organic removal rate in the mesophilic AD of WAS and DAF sludge

Table 6-13 lists the parameters measuring the quality of effluent from mesophilic AD of WAS and DAF sludge. All reactors had pH levels within the trade waste limits, yet only the reactor fed with slurry C (the lowest organic content) had effluent satisfying the trade waste criteria for CODs and TVFA. Though all the reactors exceeded the ammonia level, M-14-C and M23-C had the least ammonia concentration compared with other reactors.

Table 6-13: Effluent Quality of Mesophilic AD of Codigestion of WAS and DAF Sludge (Over the Stable Period)

Reactors	pH	CODs (mg/L)	TAN (mgN/L)	TVFAs (mg/L)
M-14-A	6.75±0.11	15,683 ±2704	1,515 ±453.8	4,587 ±1146
M-14-B	6.75±0.08	9,953 ±2851	963 ±387.7	2,949 ±1353
M-14-C	6.69±0.09	3,150 ±793	409 ±149.2	1,085 ±787
M-23-A	6.79±0.08	14,867 ±3484	1,411 ±528.9	4,340 ±1812
M-23-B	6.74±0.19	12,243 ±1617	1,144 ±259.8	3,325 ±1196
M-23-C	6.95±0.13	3,503 ±759	457 ±95.6	872 ±208
Trade Waste Limits (CWW)	**6.00-10.00**	**4000**	**200**	**1000**

Samples of digestate from all reactors were allowed to settle by gravity for four hours (see Figure 6-39). It was observed that a layer of floating scum formed in all test tubes. The thickness of the layer was proportional to the strength of the feedstock (i.e., DAF sludge slurries). This trend is consistent with that observed for mesophilic AD of DAF sludge slurries. The layer is likely caused by the lipids in the DAF sludge, which has a tendency to form floating scum (Salminen, EA et al.,2002b). Comparing the digestate from the codigestion of DAF sludge with WAS operated at a HRT of 23 days, with AD of DAF sludge slurry C (DAF sludge of low TS), operated at similar conditions (i.e., HRT of 23 days and OLR of 0.6 g VS/L.day, see Figure 6-25), it was observed that the layer for the codigestion was thicker.

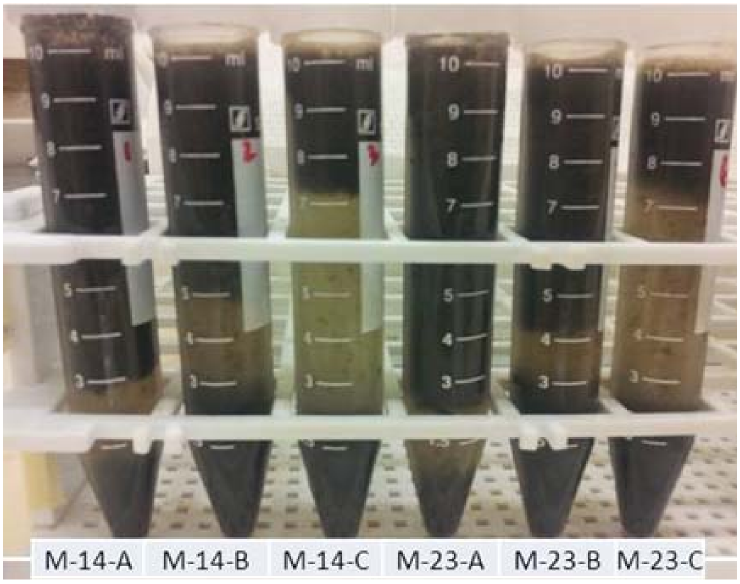


Figure 6-39: Settling conditions of codigestion of WAS and DAF sludge after mesophilic AD

Table 6-14: Summary of Mesophilic AD of Codigestion of DAF Sludge with WAS

Reactors	HRT day	OLRs		Biogas	Methane	COD Removal	VS Removal
		gCOD/gVS	g VS/L.day	mL/gVS_{added}.day			
M-14-A	14	0.26	3.85	34	21	28%	34%
M-14-B	14	0.29	2.51	39	27	20%	30%
M-14-C	14	0.20	1.05	57	38	23%	17%
M-23-A	23	0.17	2.34	41	26	30%	35%
M-23-B	23	0.16	1.53	46	29	23%	30%
M-23-C	23	0.13	0.64	86	61	27%	16%

6.2.5. **Comparison of Mesophilic AD of Different Substrates Under Semi-Continuous conditions**

A summary of the above test results can be found in Table 6 15. The main effects plots generated from Minitab show longer HRT yield higher biogas. However, the feeding material and OLR have more significant effects on biogas yield compared with the HRT. Mesophilic AD of WAS and AD of biosolids showed the highest biogas yield, and mesophilic AD of DAF sludge of low TS and codigestion of DAF sludge with WAS yielded less than average. Though mesophilic AD of DAF sludge had the lowest biogas potential yield, when codigestion was with WAS, the biogas yields improved.

Overall, OLR does not show any linear relationship with biogas yields as seen in Figure 6 40. Since those factors interact, the interaction plot (see Figure 6-41) shows a clear relation between the OLR and the biogas yield. When separating the digest material, all reactors showed increasing OLR caused decreasing in the biogas yield. The rate of biogas reduction due to increasing OLR was higher in AD materials of WAS and biosolids when compared with AD of DAF sludge and WAS and DAF sludge.

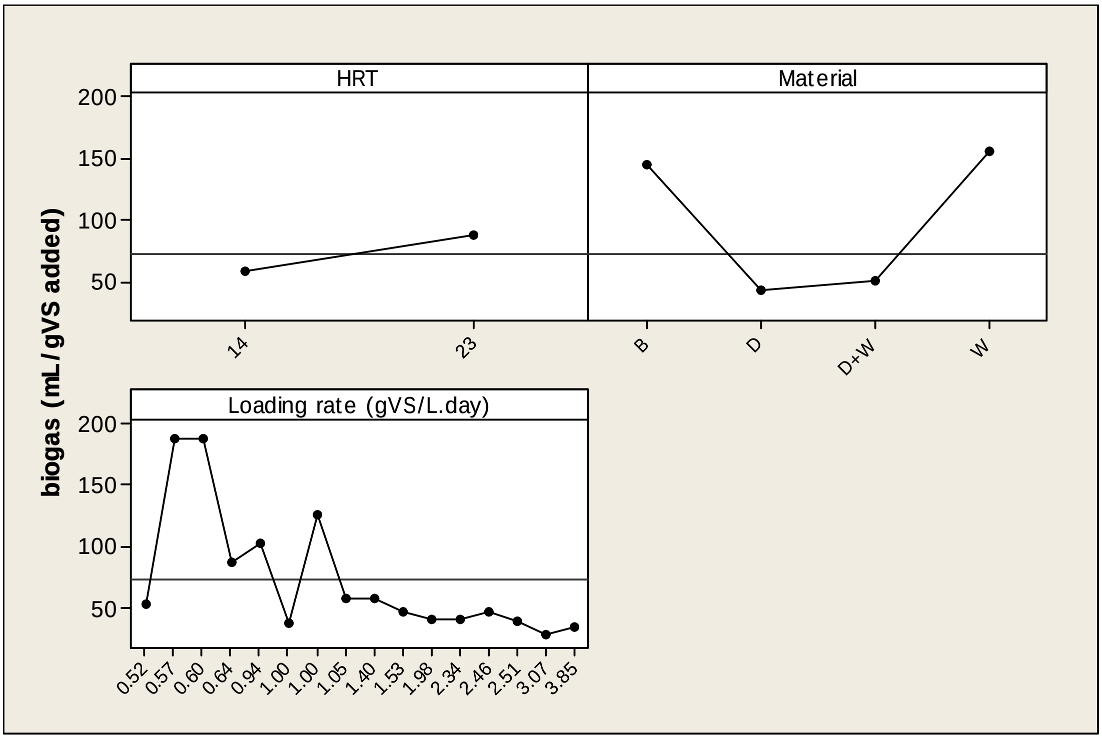


Figure 6-40: Main effect plot for biogas yield from mesophilic AD of WAS, biosolids, DAF sludge and the combinations of waste materials

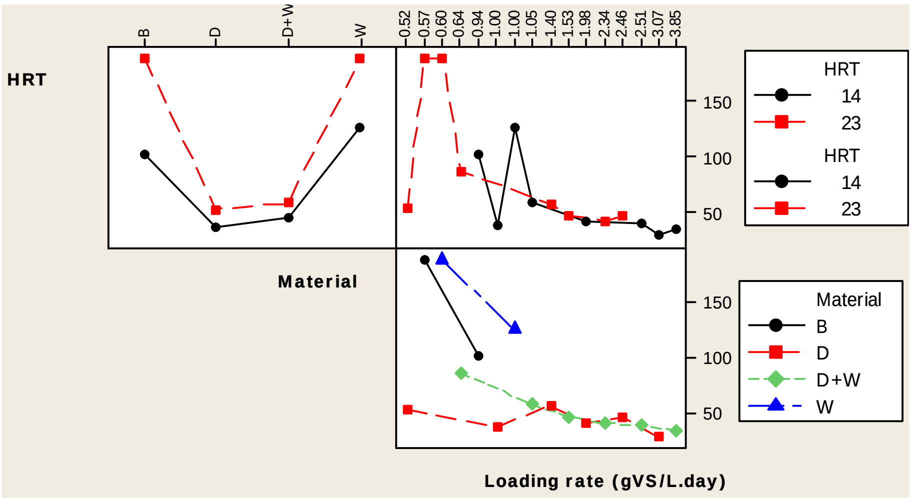


Figure 6-41: Interaction plot for biogas yield from mesophilic AD of WAS, biosolids, DAF sludge and the combinations of waste materials

Table 6-15: Summary of Results from Mesophilic AD Tests (Data During the Stabilised Period

Materials	HRT	OLRs		Biogas	Methane	COD	VS	Start-up
	day	gCOD/ gVS	g VS/L.day	mL/gVS$_{added}$.day*		Removal (%)		
Thickened WAS	14	0.2	1.0	125	78	17%	19%	Substrate to inoculum ratio according to HRT on day one and onwards
	23	0.1	0.6	188	133	22%	18%	
Diluted Biosolids	14	0.13	0.94	94	75	35%	30%	
	23	0.08	0.57	180	136	32%	29%	
Raw DAF Sludge	30	0.09	2.66	44	22	18%	35%	
DAF Sludge of Low TS	14	0.24	3.07	28	20	23%	27%	Substrate to inoculum ratio of 30:70v/v on day one and according to HRT onwards
	14	0.26	1.98	40	28	22%	37%	
	14	0.28	1.00	37	27	48%	51%	
	23	0.17	2.46	46	33	39%	27%	
	23	0.19	1.40	57	43	21%	38%	
	23	0.17	0.52	53	36	29%	40%	
	23	0.17	0.65	64	39	19%	26%	Started substrate to inoculum ratio according to HRT on day one and onwards
	30	0.16	0.50	87	54	40%	40%	
Non-polymer DAF Sludge	23	0.24	0.62	76	49	37%	41%	
DAF Sludge of Low TS	23	0.14	0.44	214	160	63%	56%	
	30	0.16	0.44	211	159	60%	50%	
	23	0.21	0.58	228	171	51%	53%	Acclimated inoculum
	30	0.19	0.58	282	213	58%	55%	
DAF Sludge + WAS	14	0.26	3.85	34	21	28%	34%	Substrate to inoculum ratio of 30:70v/v on day one and according to HRT onwards
	14	0.29	2.51	39	27	20%	30%	
	14	0.20	1.05	57	38	23%	17%	
	23	0.17	2.34	41	26	30%	35%	
	23	0.16	1.53	46	29	23%	30%	
	23	0.13	0.64	86	61	27%	16%	

6.3 Temperature Phased AD in Semi-continuous mode/experiments (TPAD)

TPAD is a two-phase digestion process, which is carried out using two reactors. The first reactor is operated at thermophilic conditions (approximately 55° C) and short HRT (e.g., two to six days), whereas, the second reactor is operated at mesophilic conditions (approximately 35°C) and longer HRT (e.g., 10 to 15 days). Compared with mesophilic AD, the first reactor provides the conditions for enhanced hydrolysis and degradation of organics such as grease trap wastes rich in fats.

According to the published literature TPAD results in a higher rate of VS reduction and better removal of pathogens with a relatively low energy input and capital cost (Ge et al.,2011b; Song et al.,2004). Riau et al., (2010) used TPAD for AD of sewage sludge and reported that VS reduction increased from 40% to 87% under a retention time of 15 days . They also found that the final biosolids were of Class A grade as the pathogens were inactivated under thermophilic conditions in the first phase reactor. Ge et al (2011b) found that AD of WAS using TPAD led to increased VS reduction from 37% to 48%. The use of TPAD improved biogas production by 30%, which was attributed to an enhancement in the hydrolysis step through the use of the thermophilic phase (Woon,2012). Woon (2011) suggested the TPAD improved 138% BMP for DAF sludge at batch conditions.

6.3.1. **TPAD from WAS**

Semi-continuous TPAD reactors were fed with the same thickened WAS used in the mesophilic AD of WAS experiment (see section 6.2.1), in order to compare the two types of AD process.

The selection of HRT was based on the published literature. Typically, the thermophilic phase HRT is three to five days, whereas the mesophilic phase HRT is 10 to 20 days (Metcalf et al.,2003). In order to investigate the effect of HRT, the first phase (thermophilic) reactors were designed to operate at a HRT of four and six days (labelled as WT4 and WT6), and the effluent from the first phase reactors was fed into the corresponding second phase (mesophilic) reactors for another 10 days (labelled as WTP10) and 17 days (labelled as WTP17), respectively. The experiment operation conditions were summarised in section 5.3.2.1.

All reactors were operated and monitored for key performance parameters over a period of 120 days. It was observed that the daily biogas production rate decreased rapidly during the first 10 days, and continued to decrease at a slower rate until day 75. The reduction indicated acclimatisation was in progress through the daily wasting and feeding. After 75 days, the change in the daily biogas yield was less variable, hence, the period from day 75 to 120 was regarded as stabilised gas production. Stabilised biogas production rates in the reactors indicated that the growth and wasting of the anaerobic microorganisms reached a balance. The daily biogas production from the first phase and combined TPAD reactors (total biogas yields from both phases) are shown in Figure: 6-42.

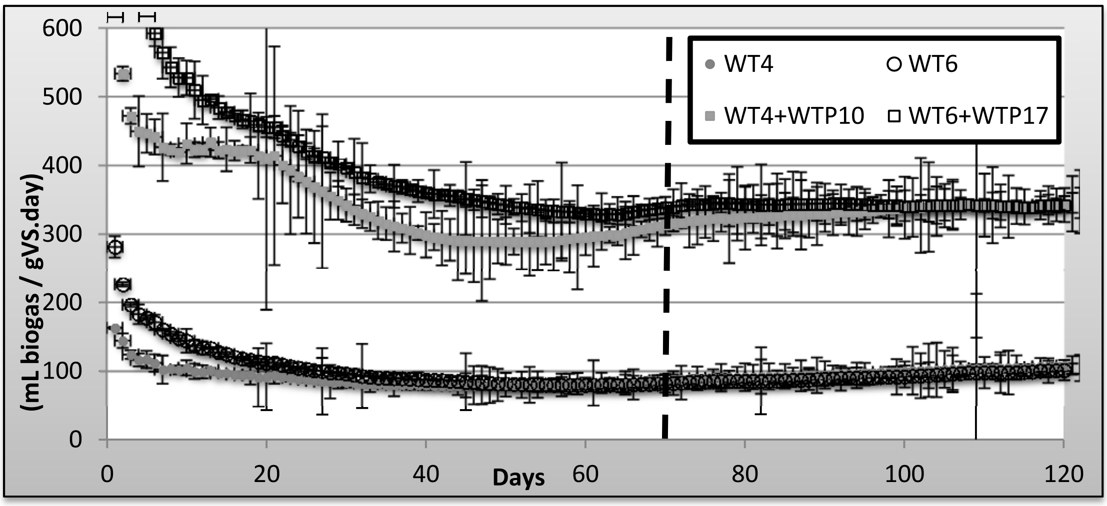


Figure: 6-42 Daily biogas yield from TPAD of WAS

Figure 6-43 shows the average daily biogas after day 75 (the stabilised period). The combined biogas yield from both the first and second phase TPAD was much higher compared with mesophilic AD under the same HRT. For example, WT4 and WTP10 yielded 235 mL/gVS$_{added}$ under TPAD, which is 88% higher than 125 mL/gVS$_{added}$ from WM14 using mesophilic AD.

The highest biogas yield of 235 mL/gVSadded for the WAS that was used in this study was obtained using TPAD at a total HRT of 14 days (four plus 10 days). Typically a yield of approximately 300 to 400 mL/gVS added has been reported for WAS generated from wastewater treatment plants. These plants usually employ conventional activated sludge processes (Davidsson et al.,2008; Luostarinen et al.,2009). The WAS used in this study was from IDEA systems (i.e., SRT of more than 20 days), which results in WAS being more stabilised compared to WAS from conventional activated sludge process. This may explain the lower biogas yield obtained for the WAS used in this study. Biogas yield from WAS using mesophilic AD and TPAD at a HRT of 14 and 23 days is shown in Figure 6-43. The theoretical biogas yield from WAS calculated based on Equation 6-1 (see section 6.2.1) was 164 mL/gVS$_{added}$, also shown in Figure 6-43. The results show that the highest biogas yield was obtained using the TPAD process. The yield obtained using TPAD at a HRT of 23 days was 223 mL/gVS$_{added}$, which is 35 mL/gVS$_{added}$ higher than the yield of 188 mL/gVS$_{added}$ using mesophilic AD at the same HRT.

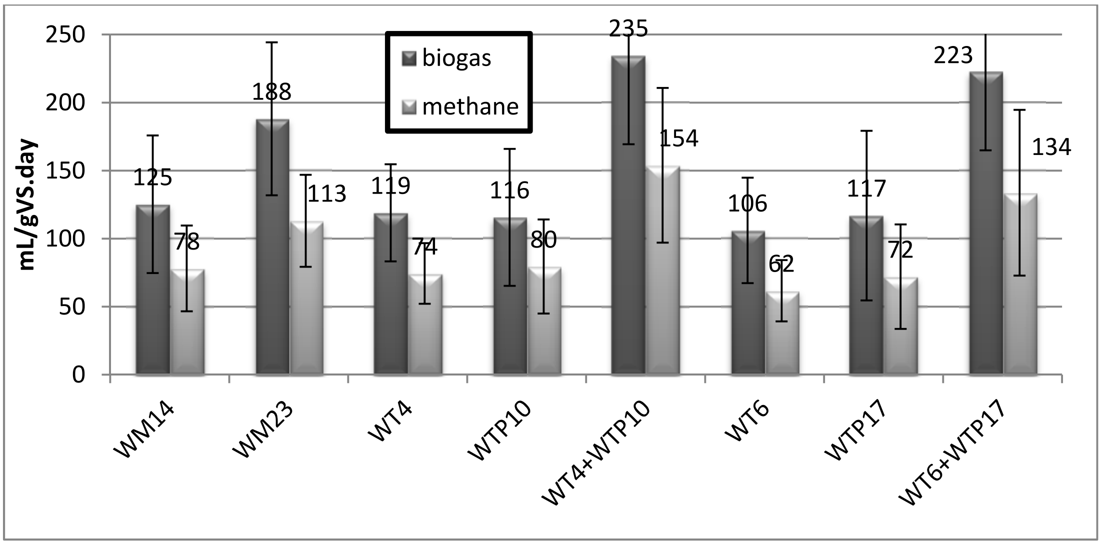


Figure 6-43: Average daily biogas and methane from mesophilic AD and TPAD of WAS

Though the TPAD reactors operated at a HRT of 23 days (i.e.WT6 and WTP17) had a longer l HRT of 9 days compared with the TPAD reactors at a HRT of 14 days (i.e. WT4 and WTP10) the biogas production was approximately 5% less. This can be due to inhibition effect of long HRT in the first phase reactor WT6. It was reported by Bolzonella(2007) that increasing the first phase reactors HRT to five days did not improve methane production. In this experiment, the first phase reactors operating at a HRT of six days had biogas yield of 106 mL/gVS.day, whereas biogas production at a HRT of four days was 119 mL/gVS.day (i.e., 10% less). It was noticed that the level of TVFAs and ammonia in the effluent from WT6 was 893 and 326 mg/L, respectively, compared with 609 and 312 mg/L, respectively, in the effluent from WT4. The high level of TVFAs at a HRT of six days indicates that the hydrolysis is proportional to the HRT. The lower biogas production in the second phase reactor at a HRT of 17 days compared to reactors at 10 days indicates that the levels of TVFAs and TAN at these conditions had an inhibitory effect on the methanogenesis microorganism present in the second phase reactors.

Figure 6-43 also shows the average daily methane production after day 75. The biogas from mesophilic reactor WM14 and WM23 had an average methane content of 62% and 60%, respectively. Among all the reactors, the highest methane content was 69% from WT10 (second phase TPAD reactor at a HRT of 10 days). This is an indication that the second phase reactors of the TPAD are achieving their goal, as one of the purposes of using TPAD is to provide a suitable environment for methanogens bacteria in the second phase reactors, hence, better quality of biogas in terms of methane content.

TPAD has the advantage of having first phase reactors to enhance the hydrolysis step, which is well recognised as the rate-limiting step in the AD process (Ge et al.,2011a, 2011b). The extent of organics solubilisation can be used as an indication of hydrolysis rate in the reactors. Evaluation of WAS solubilisation was made based on the equation proposed by Ge (2011b).

The extent of solubilisation of WAS is calculated as follows (Ge et al.,2011b)

$$\mathbf{Extent\ of\ solubilisation}\ \% = \frac{\mathbf{COD_{CH4}+COD_{s-eff}-COD_{s-in}}}{\mathbf{COD_{t-in}-COD_{s-in}}}\ \mathbf{100}\% \qquad \textbf{Equation 6-2}$$

where the COD_{CH4} is the theoretical COD equivalent of methane production during the AD process, $COD_{s\text{-}eff}$, $COD_{s\text{-}in}$, is the soluble COD in the effluent and influent, respectively, and $COD_{t\text{-}in}$ is the total COD in the influent. The theoretical COD equivalence of methane is calculated as (Isa et al. 1993):

$$\boldsymbol{1mL\ CH4\ at\ T}\text{°C} = \frac{\mathbf{273}}{\mathbf{273+}\boldsymbol{T}} \times \frac{\mathbf{760-}\boldsymbol{p}}{\mathbf{760}} \times \frac{\mathbf{1}}{\mathbf{350}} \times \mathbf{1}\boldsymbol{gCOD} \qquad \textbf{Equation 6-3}$$

where T is the temperature in degrees (°C), and p is the saturation water vapour pressure at T °C in mmHg; thus, p is 118 mmHg at 55°C and 42.2 mmHg at 35°C.

As shown in Figure 6-44, solubilisation using mesophilic AD reactors was only 2% for a HRT of both 14 and 23 days. The level of solubilisation increased from 2% to 18% and 21% using first phase TPAD reactors at a HRT of four and six days, respectively.

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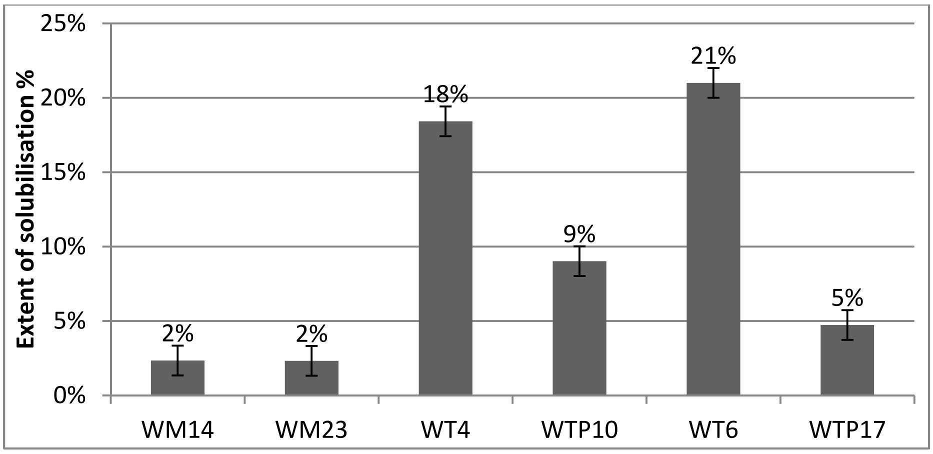

Figure 6-44: Extent of solubilisation in mesophilic AD and TPAD of WAS

Ideally, the first phase reactor serves to enhance the hydrolysis step where methanogenesis is not involved. However, the results obtained showed that methane was generated in the first phase reactors (see Figure 6-43). Separating the phases was unsuccessful; that may be due to the pH not being adjusted to be less than 5. Hence, the methanogens were still able to survive and grow. The methane content in the biogas yielded from reactor WT4 and WT6 reached 63% and 58%, respectively (averaged over a stable period). This may indicate that the HRT of four and six days are protracted for first phase TPAD. The presence of methanogenesis in first phase TPAD was also reported by Ge (2011b), who explained that as the high temperature (55°C) changing of metabolic pathways into the acetate, oxidation was thermodynamically promoted, with the acetate being oxidised to H_2 and CO_2 then subsequently converted to methane, rather than by the aceticlastis pathway (Ge et al.,2011b; Karakashev et al.,2006; SH et al.,1984). Methanosarcinaceae was found to be the dominant methanogen in acetate oxidising systems (Karakashev et al.,2006).

Figure 6-45 show that VS reduction from WTP14 was 41% higher than WM14. This is in line with the 49% higher methane yield obtained for these reactors (see Figure 6-43). Overall, TPAD shows more organic reduction in terms of removal of VS and CODt. When using mesophilic AD and TPAD treating WAS, the extended HRT from 14 to 23 days had no improvement in solids reduction, but around 5 to 6% increase in removal of CODt..

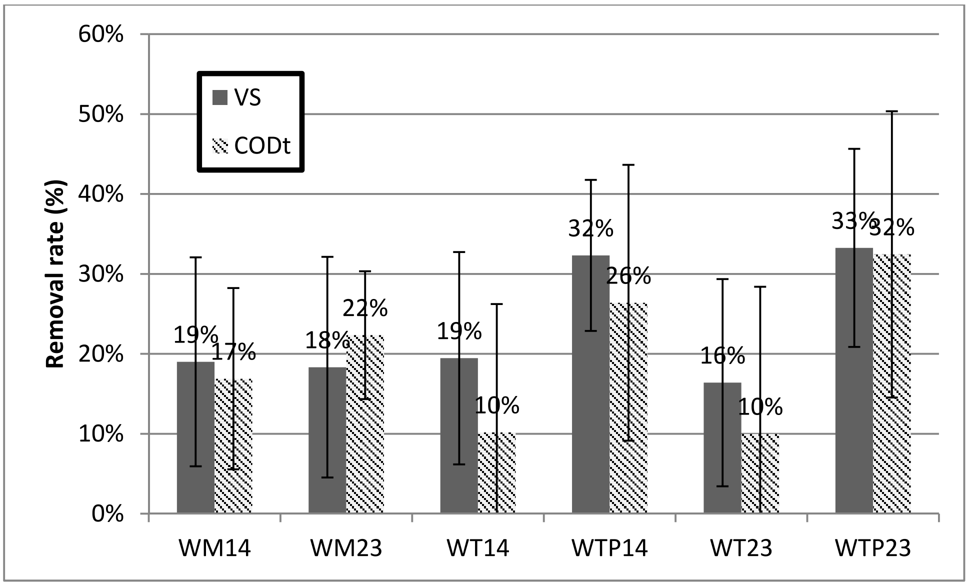

Figure 6-45: Average VS reduction from mesophilic AD and TPAD of WAS

The settling property of the AD digested sludge was investigated by allowing the effluent from the reactors to settle by gravity for four hours. Both mesophilic and second phase TPAD reactors produced poor settling sludge (see Figure 6-46). The sludge from the first phase TPAD (WT4 and WT6) had better settling compared with the others. This is because the floating layer in the effluent from AD of WAS was mainly caused by the gas trapped in the sludge that changed its density. In the first phase TPAD, the temperatures were higher (55°C) than mesophilic conditions (35°C), and the solubility of gas decreased with increasing temperature, hence, no floating sludge was observed in the first phase reactors.

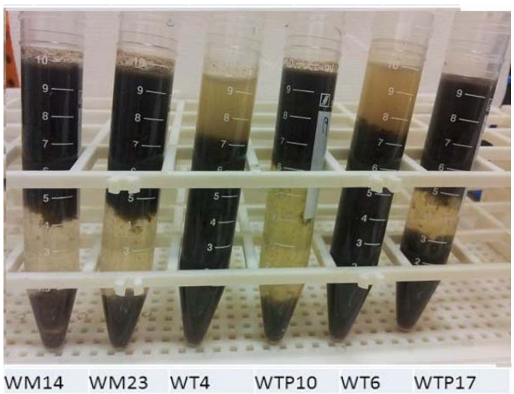

Figure 6-46: Digested sludge settling condition from mesophilic AD and TPAD of WAS

The literature has reported that higher amounts of pathogens are reduced in TPAD reactors compared with in mesophilic AD reactors compared with in mesophilic AD reactors (Ge et al.,2011b; Song et al.,2004). Microbiological testing of effluent from TPAD of WAS was conducted by the ALS group, a testing services provider. As only limited numbers of tests were granted to be sent out, the feed of the thickened WAS and the effluent from WTP17 were selected. The results showed, in Table 6-16, that the *E. coli* was reduced to 2.7 log, comparing to the feed in the reactor. *Clostridium perfringens*, which is an anaerobe growing in the absence of oxygen, was found to increase by 0.3 log rather than being removed.

Sludge treatment grades defined by EPA were clarified by the *E. coli* MPN (most probable number) of dry weight in the treated sludge. As mentioned earlier, there was only a limited number of samples being tested, so the MPN can only be assumed in the test results given here. The conversion of MPN per wet weight to MPN per dry weight is based on the equation below (USEPA,2002):

$$\frac{MPN}{g}(dry\ weight) = \frac{MPN/mL\ (wet\ weight)}{percent\ total\ solids\ (\ expressed\ as\ decimal)} \quad \textbf{Equation 6-4}$$

Hence, results in MPN/g (dw) can be calculated and shown in Table 6-16. According to the treatment grades defined by the EPA, treatment grade T1 requires less than 100 *E. coli* MPN/g dw, grade T2 has to be less than 1,000 *E. coli* MPN/g dw and grade T3 needs to go under 2,000,000 *E. coli* MPN/g dw. The effluent from WTP17 had 8392 *E. coli* MPN/g dw, which is considered as T3, though it has been treated under 55°C for six days. Using TPAD for treating WAS achieved treatment Grade T3.

Table 6-16 Microbiological Testing of Effluent from TPAD of WAS

Analysis - anolyte	Thickened WAS		WTP17		Reduction
Unit	orgs/100mL	MPN/g(dw)	orgs/100mL	MPN/g(dw)	log
Somatic Coliphage DA - Somatic Coliphage Double Agar	9,400,000	4,454,976	70,000	48,951	2.1 log
Colilert (2000) - *E. coli* MPN Colilert	**6,500,000**	**3,080,569**	**12,000**	**8,392**	**2.7 log**
Clostridia MF - Sulphite reducing Clostridia (Spores)	12,000,000	5,687,204	1,100,000	769,231	1.0 log
Clostridia MF - Clostridium perfringens	600,000	284,360	1,100,000	769,231	-0.3 log

Table 6-17: Summary of Experimental Result from TPAD of WAS

Reactors	HRT (day)		OLRs		Biogas	Methane	COD	VS	Ammonia	TVFAs
	First	Second	gCOD/gVS.d	gVS/L.d	mL/gVS$_{added}$.day		Removal (%)		mg/L	
WT4	4		0.55	0.90	235	154	26%	32%	312±30	609±448
WTP10		10	0.26						453±43	184±197
WT6	6		0.35	0.55	223	134	32%	33%	326±32	893±566
WTP17		17	0.16						424±28	165±107

6.3.2. **TPAD from Biosolids**

Mesophilic AD of biosolids has been discussed in section 6.2.2. The results showed biosolids have a potential for biogas production, where a yield of 189 mL/gVS$_{added}$ was obtained. In order to compare the biogas potential from biosolids in mesophilic AD, the same samples were used for this TPAD experiment. Diluted biosolids had a concentration of 13-17gTS/L.

As the results from TPAD of WAS indicated a HRT of four and six days has a negative effect on the AD process, in this experiment, the HRT of the first phase reactors of the TPAD process was shortened to 2 days. The first phase reactors were fed with raw biosolids and operated under a HRT of 2 days. These reactors were labelled as BT2. The second phase TPAD reactors were operated under a HRT of 12 and 21 days (i.e., at OLRs of 0.9 g VS/L.day and 0.6 g VS/L.day, respectively). These reactors were labelled as BTP12 and BTP21, respectively (see section 5.3.2.2). By this setup, the total HRT of TPAD is 14 and 23 days, which is same as the HRT applied for mesophilic AD of biosolids, and reactors BM14 and BM23.

The combined daily biogas production from both phases of TPAD shows more stabilised yield after approximately 25 days (see Figure 6-47), which is similar to the mesophilic AD (see Figure 6-12). Therefore, the average daily biogas yield did not include the first 25 days to eliminate the effect of the start-up period.

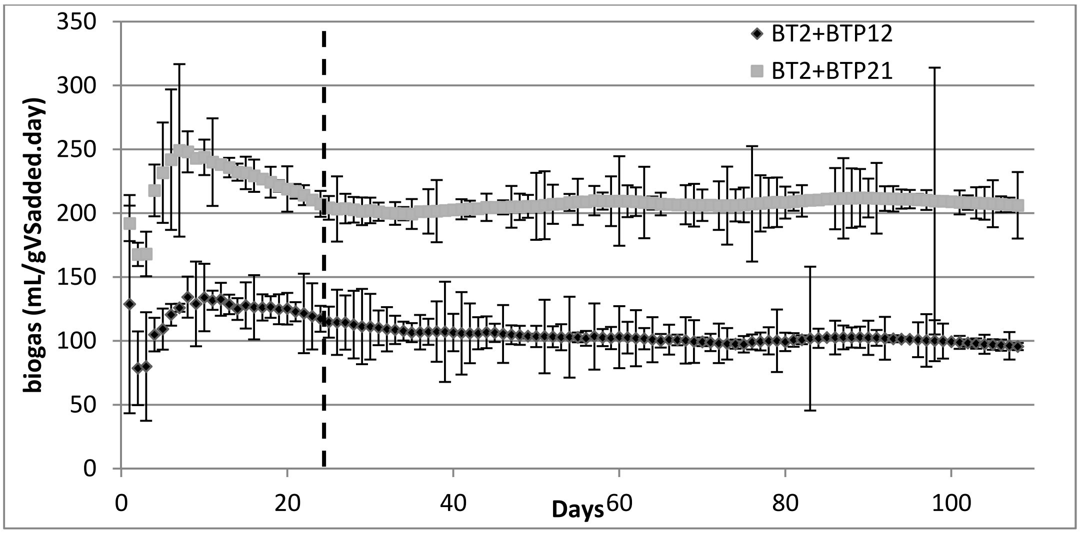

Figure 6-47: Daily biogas yield from TPAD of biosolids

Overall, the reactors operated at a longer HRT (23 days) had a higher daily biogas yield (see Figure 6-48). It was observed that using mesophilic AD, the biogas yield at a HRT of 23 days was 85% higher compared to that at a HRT of 14 days. Under the same HRT of 23 days, biogas from TPAD (BT2+BTP21) yielded 15% higher than BM23. Conversely, TPAD did not improve the biogas yield when the total HRT was 14 days. Overall, the increase of biogas yield by TPAD was not as significant as the improvement at the TPAD of WAS.

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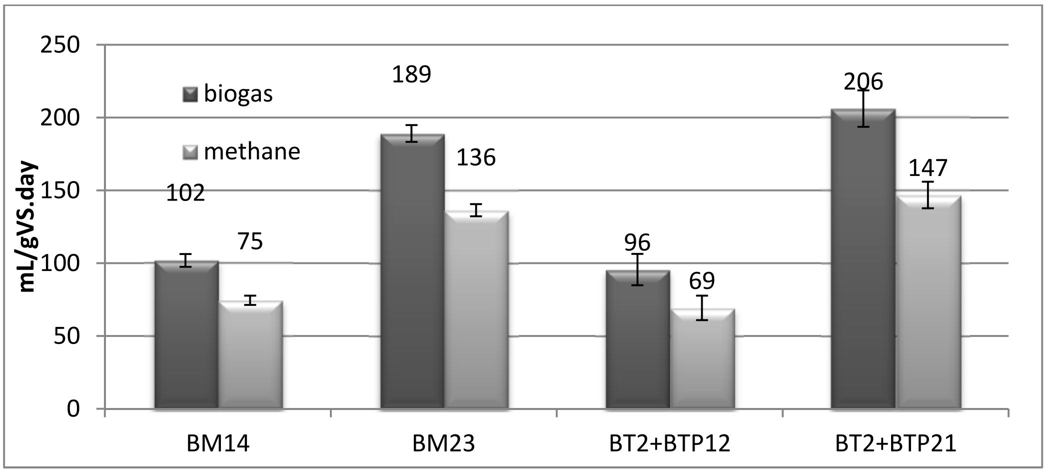

Figure 6-48: Average biogas and methane yields from mesophilic AD and TPAD of biosolids

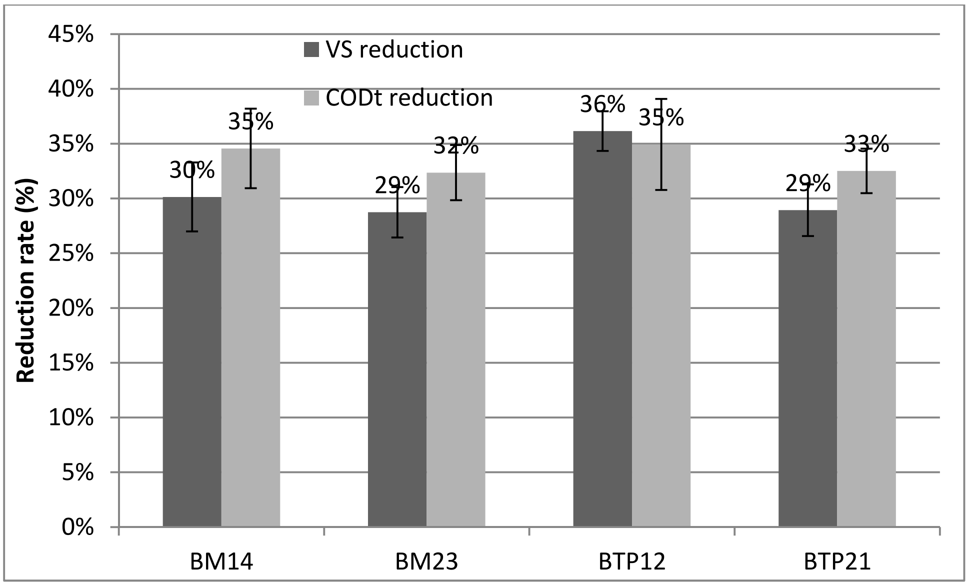

Figure 6-49: Organic removal in the biosolids AD reactors

During the stable period (after day 25), the VS content was reduced by 29% and 30% in BM14 and BM23, respectively (see Figure 6 14). Compared with the VS reduction in TPAD of biosolids, that was 36% and 29% under a HRT of 14 and 23 days correspondingly. The reduction percentage is quite similar though using a different process. The result obtained from mesophilic AD and TPAD continuous experiments matches the same trend of batch tests, that the TS reduction of 40% in biosolids samples were recorded in a batch test.

Other parameters for measuring the organic removal rate is the total COD, which resulted in a 35% reduction in reactor BTP12 and 33% in reactor BTP21. Likewise, the TPAD showed similar organic reduction with mesophilic AD in terms of CODt removal. This matches the results that biogas was not enhanced as much as AD of WAS when using TPAD.

Over time, the effluent from BTP12 and BTP21 had average TVFAs (ranging from 76 to 125 mg/L) and CODs (varying from 1,100 to 627 mg O_2/L) concentration under the trade wastes limits of 1,000 mg acetic acid/L and 4,000 mg O_2/L, respectively. The only parameter that exceeded the trade waste limit of 200 mg N/L was the ammonia. Effluent from all the reactors varied from 383 mg N/L to 499 mg N/L, which is excess ammonia of 183 to 299mg N/L (see Figure 6-50).

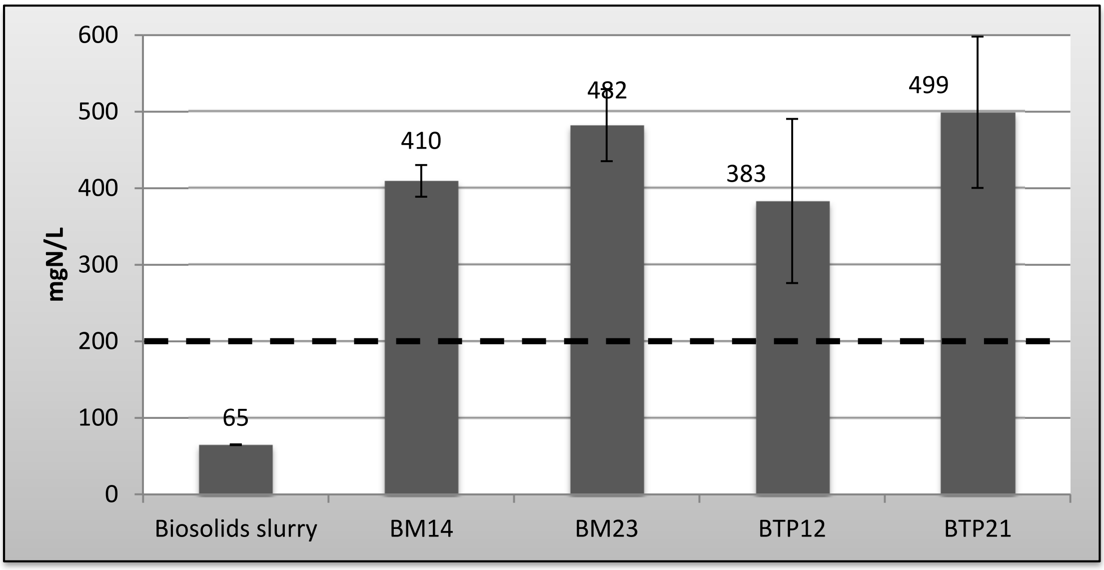

Figure 6-50: TAN in mesophilic AD and TPAD of biosolids

The digested sludge was tested for its gravity settling characteristic by allowing the effluent from the reactors to settle by gravity for four hours. The digested biosolids showed poor settling regardless of the AD process used for digestion; that is, no separation of solids from supernatant by gravity was observed (see Figure 6 51). This may indicate that the sludge from both mesophilic AD and TPAD of biosolids under conditions tested would require further processing for dewatering. Further research maybe carried out to testify it.

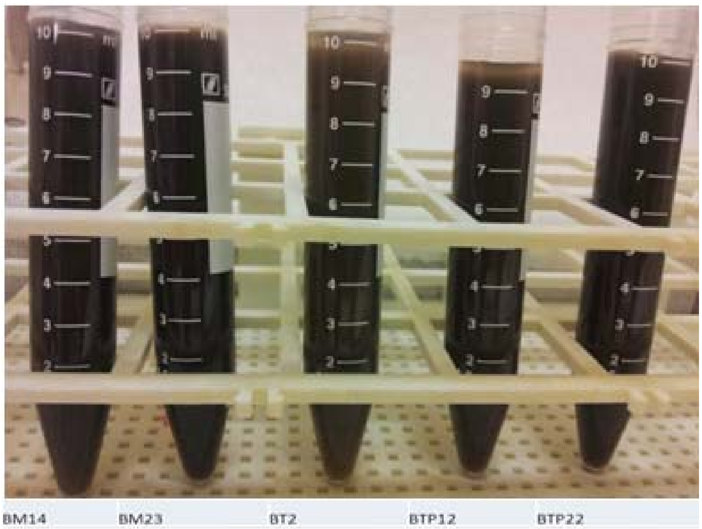

Figure 6-51: Digested Biosolids settling conditions (mesophilic and TPAD)

Table 6-18: Summary of TPAD of Biosolids Experimental Result

Reactors	HRT(day)		OLRs		Biogas	Methane	COD	VS	Ammonia	TVFAs
	First	Second	gCOD/gVS	g VS/L.day	mL/gVS_{added}.day		Removal (%)		mg/L	
BT2	2		1.23	0.94	96	69	35%	36%	409±38	970±99
BTP12		12	0.21						383±107	125±62
BT2	2		1.23	0.57	206	147	33%	29%	409±38	970±99
BTP21		21	0.10						499±99	76±6

6.3.3. TPAD from DAF Sludge

The potential of using TPAD for the treatment of DAF sludge was assessed and compared with mesophilic AD. The results obtained are discussed in this section. Woon (2012) reported biogas yield of 18 and 100 mL/gVSadded were obtained using TPAD and mesophilic AD, respectively. Although biogas yield reported by Woon (2012) was higher than mesophilic AD, the yield in general was lower than biogas yield reported for typical wastewater treatment sludge (i.e., WAS). Further, the VS and TS removal were lower than VS reported for WAS. Therefore the aim this experiment was to assess TPAD for DAF sludge of different TS concentrations, hence, different initial TVFAs and TAN.

6.3.3.1. *Raw DAF sludge*

TPAD of raw DAF was tested under a HRT of eight days (labelled as DT8) for first phase and 15 & 20 days for second phase (labelled as DTP15 and DTP20). The treatment of this raw DAF sludge using mesophilic AD was discussed in section 6.2.3.1. Woon (2012) reported that TPAD at a HRT of 28 days (first phase eight days, second phase 20 days) showed the best performance for the treatment of raw DAF sludge in terms of biogas yield. Therefore, this study investigated the performance of TPAD for the AD of DAF sludge of different TS concentrations at a HRT of 28 days, where the first and second phase were operated at a HRT of eight and 20 days, respectively. As discussed in section 6.2.3.1, during the test period there were variations in the characteristics of the DAF sludge samples collected (e.g., the VS ranged from 48 to 151 gVS/L). Figure 6-17 shows the VS concentration of the DAF sludge sample.

The normalised daily biogas production from all reactors is shown in Figure 6-52. The trend in biogas yield showed four distinct periods similar to the trend observed for mesophilic AD of DAF sludge (see section 6.1.3). The first period is the start-up period, where biogas production showed large variation. Biogas production from the reactors operated at second phase reactors during the period from day 20 to day 60 showed little variation (i.e., standard deviation was less than 10%). Similarly, during the period of day 100 to later, little variation in biogas production from the reactor was observed. This period is referred to as period (4).

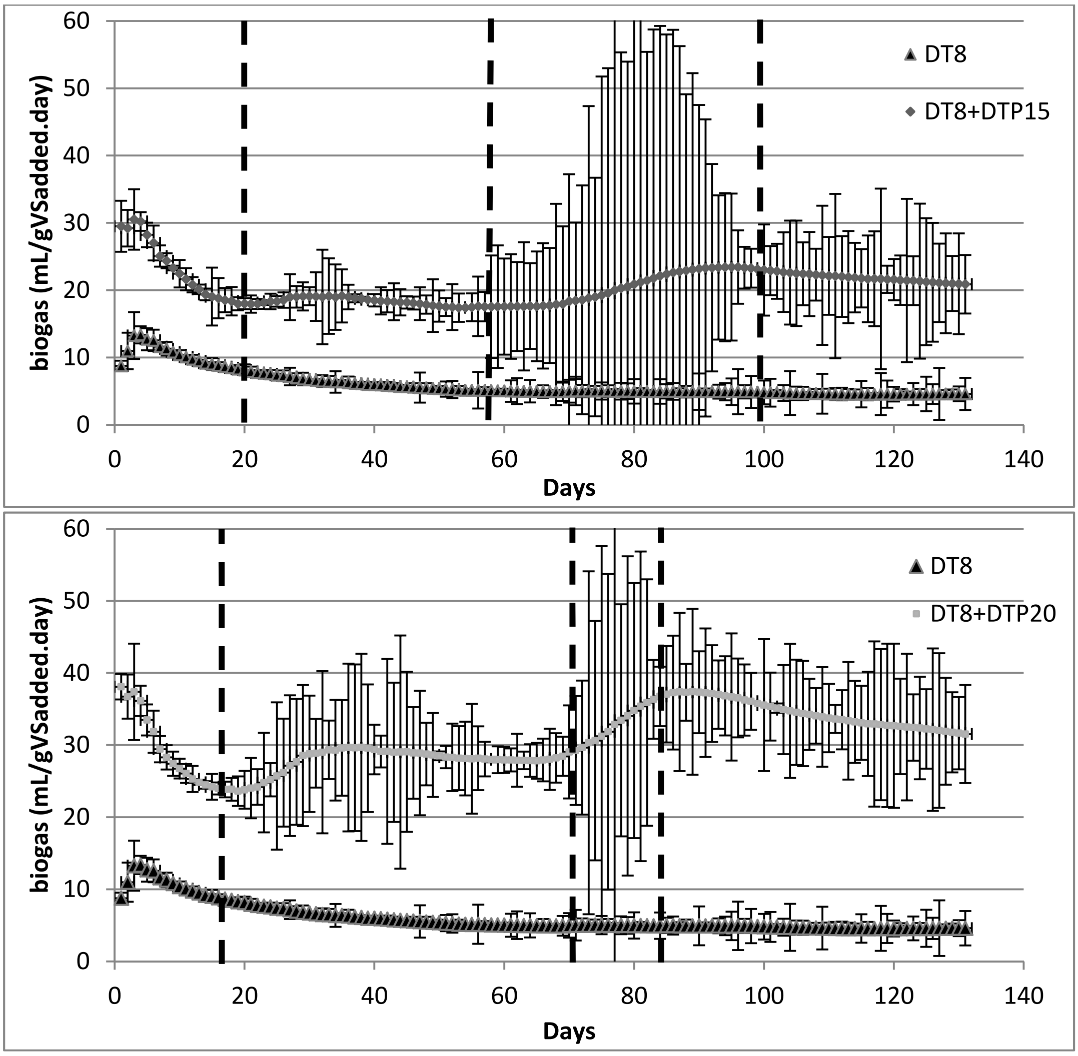

Figure 6-52: Daily biogas yield from TPAD of raw DAF sludge

average daily yield of biogas and methane from mesophilic AD and TPAD is shown in Figure 6-53. Compared with those two processes, TPAD here did not improve the biogas yield. As Hejnfelt (2009) reported, animal waste contains high ammonia loads and causes the susceptibility of the thermophilic digestion to ammonia, so the first phase of TPAD reactors that ran under thermophilic conditions caused higher ammonia levels. Table 6-19 shows the ammonia concentration of effluent in the reactors, confirming the ammonia level was higher in the TPAD reactors in comparison to mesophilic AD.

In terms of organic removal, TPAD showed better VS and CODt removal compared with mesophilic AD, which agrees with the literature that TPAD enhances the VS removal(Ge et al.,2011b; Song et al.,2004). With longer HRT (e.g., DTP20), the VS reduction improved from 36% to 52% compared with DTP15.

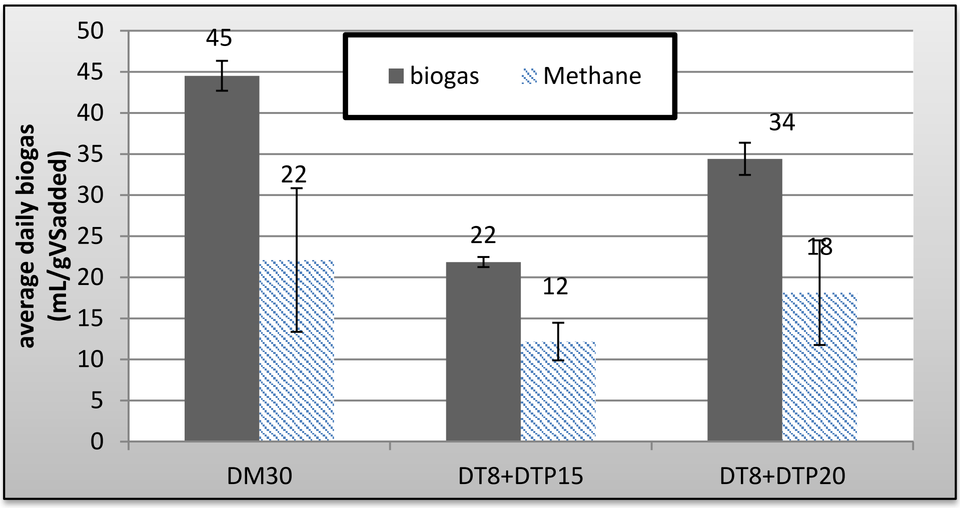

Figure 6-53: Average daily biogas and methane yield from mesophilic AD and TPAD of DAF sludge over the period (4)

As mentioned earlier, the ammonia level was higher in the TPAD reactors, and so was the TVFAs concentration. This indicates a better degradation of complicated organic matters into a simple format such as acetic acid. However, more ammonia was released as by-products at mean time, and due to the inhibition effect of high ammonia concentration on methanogenesis bacteria, the final stage of conversion of the TVFAs into methane gas was constrained.

Table 6-19: Performance of Mesophilic AD and TPAD of DAF Sludge Over Period (4)

Reactors	VS reduction	COD reduction	pH	TVFAs (mg/L)	TAN (mg/L)
DM30	18±13%	35±8%	6.88±0.16	11,072±67	1240±678
DTP15	36±4%	33±27%	6.64±0.13	12,688±1,951	1751±586
DTP20	52±4%	46±4%	6.73±0.26	12,026±3,867	1859±882
Inhibition limits			pH<7	TVFAs>7g /L (Hejnfelt et al.,2009)	TAN>1400mg/L (Jarrell et al.,1987)

Table 6-20: Summary of Experimental Result from TPAD of raw DAF Sludge

	HRT(day)		OLRs		Biogas	Methane	COD	VS
	first	second	gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day		Removal (%)	
DT8	8		0.77	3.47	22	12	33%	36%
DTP15		15	0.25					
DT8	8		0.77	2.85	34	18	46%	52%
DTP20		20	0.24					

6.3.3.2. *DAF sludge of Low TS*

Tested at the same time as HM23 and HM30, which were the mesophilic AD of DAF sludge of low TS (discussed in section 6.2.3.2). Therefore, the same feeding materials were used in that experiment to eliminate the variation of waste samples.

The first phase reactors were operated at a HRT of four and six days (labelled as HT4 and HT6), and the effluent from the first phase reactors was then fed into the corresponding second phase reactors for another 10 days (labelled as HTP10) and 17 days (labelled as HTP17), respectively. A similar start-up approach was used as the substrate to inoculum ratio was based on the HRT from day one onwards.

Figure 6-54 showed the daily biogas yield from mesophilic AD and TPAD of DAF sludge of low TS started with the substrate to inoculum ratio according to HRT on day one onwards. The biogas yield increased over the start-up period rather than decreasing, except the HT4+HTP10. After a turning point, the biogas yield started to decrease and become more stable, but HT4+HTP10 reached the stable period right after the initial decrease of biogas yield from day one to day 10. After around day 15, HM23 became stable. Both HM30 and HT6+HTP17 reached stable status around day 70.

Though running at the same total HRTs, HT6+HTP17 and HM23 showed quite different behaviour in terms of the day reaching the stable status (Figure 6-54). Instead, the reactor HM30 displayed more alike trends with HT6+HTP17 as the stabilization reached around similar time. Overall, all reactors became stable after 80 days.

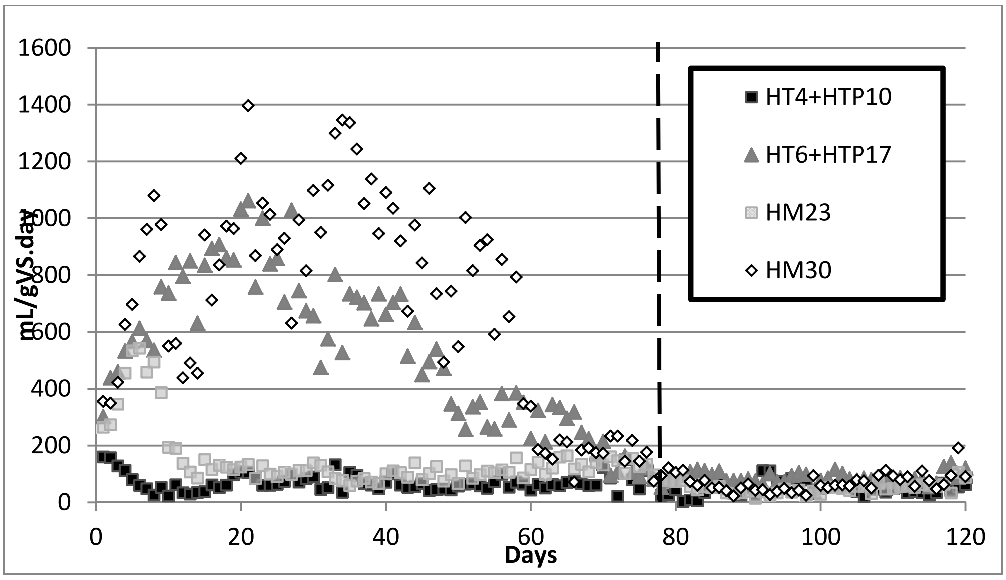

Figure 6-54: Daily biogas yield from mesophilic AD and TPAD of DAF sludge of low TS

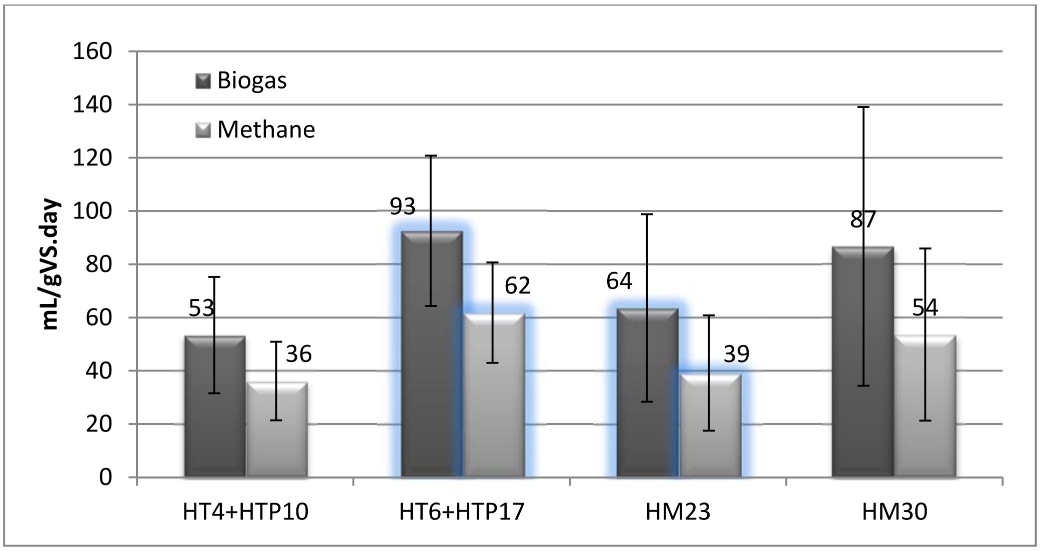

Figure 6-55: Average daily biogas yield from mesophilic AD and TPAD of DAF sludge of low TS (after day 80)

For the convenience of data analysis, the average daily biogas and methane yield used data after day 80, shown in Figure 6-55. Here, the TPAD of DAF sludge of low TS showed improvement of biogas yield from 64 mL/gVS$_{added}$ to 93 mL/gVS$_{added}$ at a same total HRT of 23 days. Overall, longer HRT yielded higher biogas, and it was valid for both mesophilic AD and TPAD under testing range. Though HM30 had a longer HRT of 30 days under mesophilic AD, TPAD under a HRT of 23 days (HT6+HTP17) yielded 7% higher biogas.

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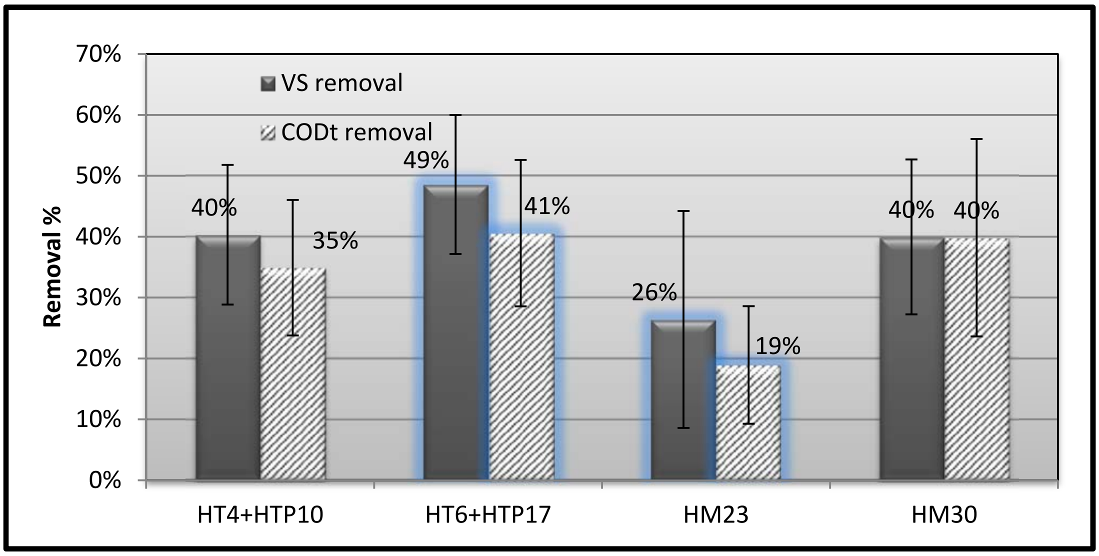

Figure 6-56: Organic removal from mesophilic AD and TPAD of DAF sludge of low TS

Similar to raw DAF sludge, TPAD showed better VS and CODt removals compared with mesophilic AD. Under the same HRT of 23 days, the VS reduction increase from 26% to 49% when using the TPAD rather than mesophilic AD. With longer HRT (e.g., HM30), the VS reduction improved from 26% to 40% compared with HM23. The relationship in between the HRT and organic reductions was also applicable to TPAD of DAF sludge of low TS.

With the AD of DAF sludge of low TS, TPAD not only increased the organic reduction, but also improved the biogas yields in comparison to mesophilic AD. However, AD of raw DAF sludge showed opposite results. As explained in pervious sections, the amount of ammonia released by degradation of nitrogenous matter was very high and caused an inhibitory effect on the methanogenesis step. TPAD of raw DAF sludge improves the degradation rate compared with mesophilic AD; consequently, higher amounts of ammonia were released and in that case exceeded the limits of causing inhibitions. By mixing DAF sludge with water as the feed into the AD reactors, the concentration of nitrogenous matter was much lower, thus the released ammonia amount would be less, and the results showed the ammonia level was well below the limits of 1,400 mgN/L inhibiting the AD process (see Table 6-21).

Conversely, the ammonia and TVFAs were under the AD inhibition limits, but exceeded the tradewaste limit, which meant the quality of the supernatant was not good enough to be disposed into drainage without further treatment to reduce the level below the limits, otherwise the cost of disposal trade waste would rise.

Table 6-21: Effluent Quality of Mesophilic AD and TPAD of DAF Sludge of Low TS (During the Stabilised Period)

Reactors	pH	CODs(mg/L)	TVFAs (mg/L)	TAN (mg/L)
HM23	7.09±0.10	2907±2059	1676±566	468±142
HM30	7.18±0.09	2543±1840	1457±75	329±114
HTP10	7.00±0.20	3105±2300	1370±142	346±52
HTP17	7.17±0.17	1937±1269	1331±568	341±87
Trade Waste Limits (CWW)	6-8	1000	1000	200

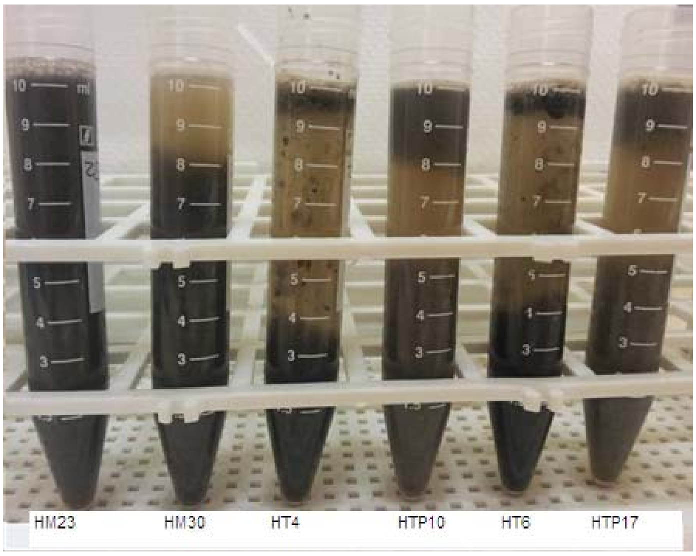

Figure 6-57: Settling condition of mesophilic AD and TPAD of DAF sludge of low TS

The digested sludge was tested for its settling property by allowing the effluent from the reactors to settle by gravity for four hours. Poorly settling sludge was found from second phase TPAD of DAF sludge of low TS, whereas the mesophilic AD reactor that ran under a HRT of 30 days showed better settling as there was no floating sludge on top (see Figure 6-57).

Microbiological testing of effluent from TPAD of DAF sludge of low TS was conducted by the ALS group. As only a limited number of tests were granted to be sent out, the feed of DAF sludge of low TS and the effluent from HTP17 were selected. The results, shown in Table 6-25, indicate that *E. coli* was reduced to 2.7 log compared to the feed into the reactor, which had a same reduction of *E. coli* in the WTP17 (TPAD of thickened WAS under a total HRT of 23 days).

Sludge treatment grades defined by the EPA were clarified by the *E. coli* MPN of dry weight in the treated sludge. The conversion of MPN per dry weight is calculated by the equation given in section 6.3.1. Hence, results in MPN/g (dw) can be found in Table 6-24. According to the treatment grades defined by the EPA, treatment grade T1 requires less than 100 *E. coli* MPN/g dw, grade T2 has to be less than 1,000 *E. coli* MPN/g dw and grade T3 needs to be under 2,000,000 *E. coli* MPN/g dw. The effluent from HTP17 had 11,841 *E. coli* MPN/g dw, which is considered T3, though it has been treated under 55°C for six days. Using TPAD for treating DAF sludge of low TS achieved treatment grade T3.

Table 6-22: Microbiological Testing of Effluent from TPAD of DAF Sludge of Low TS

Analysis - anolyte	Thickened WAS		HTP17		Reduction
Unit	orgs/100mL	MPN/g(dw)	orgs/100mL	MPN/g(dw)	log
Somatic Coliphage DA - Somatic Coliphage Double Agar	14,600,000	10,209,790	2,200	1,532	3.8log
Colilert (2000) - *E. coli* MPN Colilert	**8,700,000**	**6,083,916**	**17,000**	**11,841**	**2.7 log**
Clostridia MF - Sulphite reducing Clostridia (Spores)	2,500,000	1,748,252	210,000	146,276	1.1 log
Clostridia MF - Clostridium perfringens	830,000	580,420	84,000	58,510	1.0 log

Table 6-23: Summary of Experimental Result from TPAD of DAF Sludge of Low TS

Reactors	HRT(day)		OLRs		Biogas	Methane	COD	VS
	First	Second	gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day		Removal (%)	
HT4	4		0.69	1.07	53	36	35%	40%
HTP10		10	0.41					
HT6	6		0.46	0.65	93	62	41%	49%
HTP17		17	0.30					

6.3.3.3. *Non-polymer DAF sludge*

TPAD of non-polymer used the same feeding discussed in section 6.2.3.3 and was carried out at the same time of the mesophilic AD of non-polymer DAF sludge. The first phase reactors were operated at a HRT of four and six days (labelled as NT4 and NT6), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as NTP10) and 17 days (labelled as NTP17), respectively. This is the same as the start-up of mesophilic AD of non-polymer DAF sludge that the substrate to inoculum ratio was based on the HRT on day one onwards, and then daily feed and waste.

Figure 6-58 shows the daily biogas yield from mesophilic AD and TPAD of non-polymer DAF and DAF sludge of low TS. A very different trend of start-up period was observed compared with the continuous reactor started with a substrate to inoculum ratio of 30:70v/v on day one and then a ratio according to its HRT onwards, but similar to the mesophilic AD of non-polymer DAF sludge substrate to inoculum ratio based on the HRT on day one onwards. The biogas yield increased over the start-up period, then started to decrease after the peak, and became more stable. NT4+NT10 were the first set of reactors that became stable around day 40. NM23 and NT6+NT17 reached stable status around day 70.

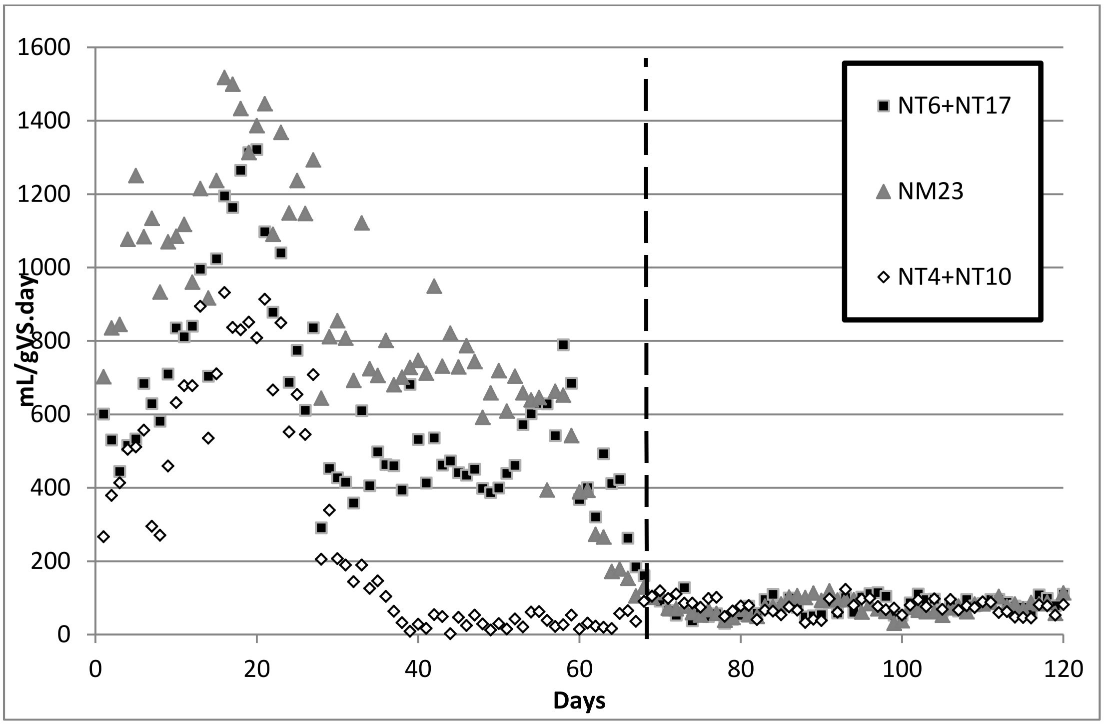

Figure 6-58: Daily biogas yield from mesophilic AD and TPAD of non-polymer DAF sludge

When comparing the average daily biogas yield, average value was taken at the stable period (after day 70) when all reactors became stabilised. Figure 6-59 shows mesophilic AD and TPAD of non-polymer DAF sludge. Unlike AD of other materials, the HRT and the TPAD/mesophilic AD had insignificant effects on the biogas yield. Regardless of the conditions, biogas yield from the non-polymer ranged from 75-79 mL/gVS$_{added}$ within the tested ranges.

Akin with the biogas yield, the organic reduction was found at 41 to 46% for VS removal and 37 to 42 % for CODt removal, so that the influence of the HRT and AD process was minor (see Figure 6-60).

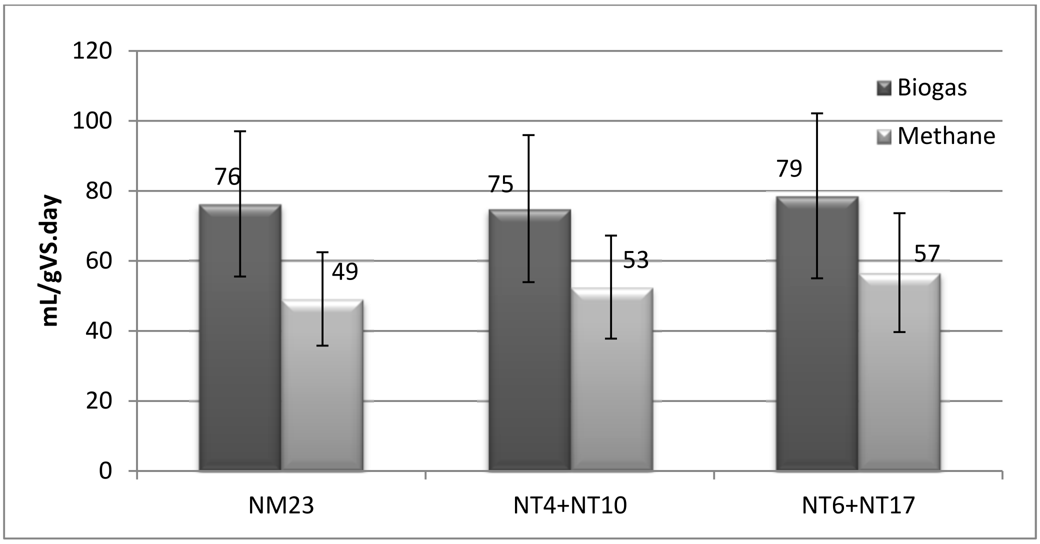

Figure 6-59: Average daily biogas yield from mesophilic AD and TPAD of non-polymer DAF sludge (after day 70)

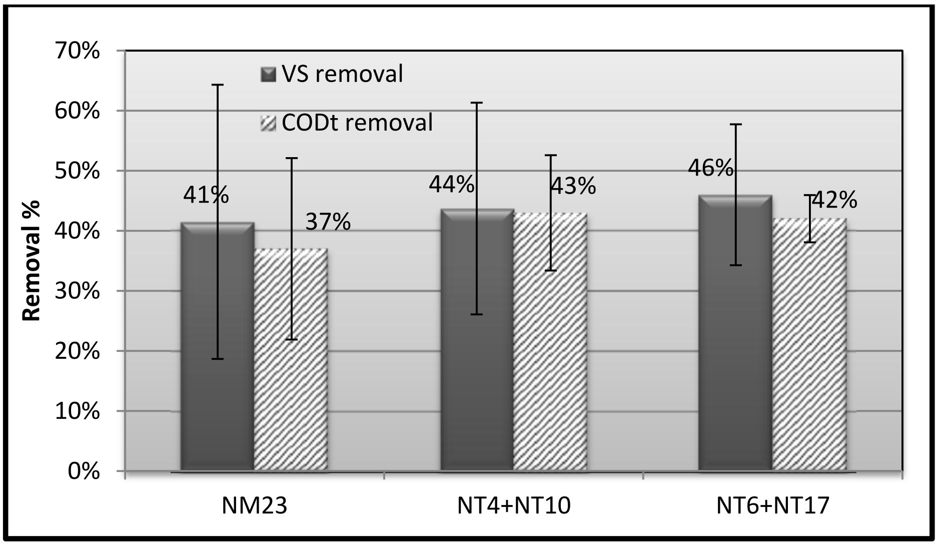

Figure 6-60: Organic removal from mesophilic AD and TPAD of non-polymer DAF sludge

When considering the effluent quality, the CODs, TVFAs and ammonia of effluent from mesophilic AD reactors exceeded the trade waste limit, but less than the inhibition limits reported by the literature. Conversely, though effluent from TPAD reactors also had the ammonia and CODs over the trade waste limits, the TVFAs were under the acceptable level. Overall, the quality of the effluent from TPAD reactors was better than from mesophilic AD reactors. For instance, the CODs from NTP17 was around 460 mg/L lower than the NM23 under the same total HRT. Table 6-24 lists some trade waste parameters.

Table 6-24: Effluent quality of Mesophilic AD and TPAD of Non-polymer DAF sludge (Over the Stable Period)

Reactors	pH	CODs(mg/L)	TVFAs (mg/L)	TAN (mg/L)
NM23	6.98±0.05	1935±1354	1191±203	315±76
NTP10	6.73±0.23	1801±1402	830±165	249±84
NTP17	6.41±0.20	1471±1133	889±203	325±64
Trade waste limits (CWW)	6-8	1000	1000	200

In this experiment, the advantage of TPAD did not reflect on the biogas yield and organic reduction, but the effluent quality and the sludge settling were better than mesophilic AD of non-polymer DAF sludge. Figure 6-61 shows the solids in the effluent of mesophilic AD reactor suspended with liquid and not settled by gravity, whereas the solids in the effluent of TPAD reactors settled in the bottom with a floating layer, meaning that superannuant was able to be separated from the sludge without further mechanic force

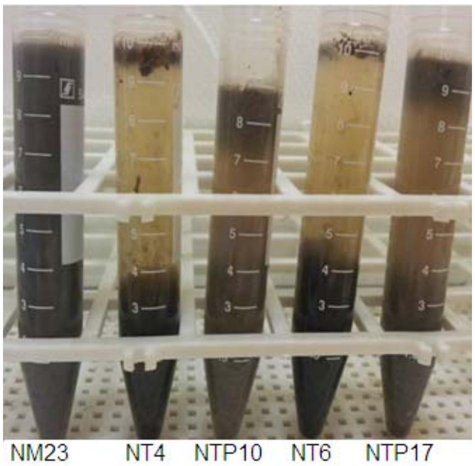

Figure 6-61: Settling condition of mesophilic AD and TPAD of non-polymer DAF sludge

Microbiological testing of effluent from TPAD of non-polymer DAF sludge was conducted by the ALS group. As only a limited number of tests were granted to be sent out, the feed of non-polymer DAF sludge and the effluent from NTP17 were selected. The results showed, see Table 6-25, that *E. coli* was reduced to 3.3 log compared to the feed into the reactor, which was higher than the reduction of *E. coli* in the WTP17 (TPAD of thickened WAS under a total HRT of 23 days) and HTP17 (TPAD of DAF sludge of low TS under a total HRT of 23 days).

Sludge treatment grades defined by EPA were clarified by the *E. coli* MPN of dry weight in the treated sludge. The conversion of MPN per dry weight is calculated by the equation given in section 6.3.1. Hence, results in MPN/g (dw) can be converted in Table 6-25. According to the treatment grades defined by the EPA, treatment grade T1 requires less than 100 *E. coli* MPN/g (dw), grade T2 has to be less than 1,000 *E. coli* MPN/g (dw) and grade T3 needs to be under 2,000,000 *E. coli* MPN/g dw. The effluent from NTP17 had 119 *E. coli* MPN/g dw, which is considered T2, which has a higher grade than WTP17 and HTP17.

E. coli in the non-polymer DAF sludge was lower than the thickened WAS and DAF sludge of low TS, and resulted in the effluent from NTP17 having much lower *E. coli* compared to effluent from the AD of other substrates. This may be due to the process of the non-polymer DAF sludge being prepared in the lab.

Table 6-25: Microbiological Testing of Effluent from TPAD of Non-polymer DAF Sludge

Analysis - anolyte	**Non-polymer DAF sludge**		**NTP17**		**Reduction**
Unit	orgs/100mL	MPN/g(dw)	orgs/100mL	MPN/g(dw)	log
Somatic Coliphage DA - Somatic Coliphage Double Agar	330,000	222,973	200	199	3.2 log
Colilert (2000) - ***E. coli*** MPN Colilert	**240,000**	**162,162**	**120**	**119**	**3.3 log**
Clostridia MF - Sulphite reducing Clostridia (Spores)	170,000	114,865	65,000	64,681	0.4 log
Clostridia MF - Clostridium perfringens	<10,000	n/a	<1,0000	n/a	n/a

Table 6-26: Summary of Experimental Result from TPAD of Non-polymer DAF Sludge

Reactors	HRT(day)		OLRs		Biogas	Methane	COD	VS
	First	Second	gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day		Removal (%)	
NT4	4		0.78	1.01	75	53	43%	44%
NTP10		10	0.49					
NT6	6		0.53	0.62	79	57	42%	46%
NTP17		17	0.29					

6.3.4. **TPAD from WAS and DAF sludge in codigestion mode**

Since TPAD of DAF sludge of low TS has improved the biogas yield and organic removal compared with mesophilic AD, the rational of enhancement on hydrolysis in the thermophilic first phase should also be applicable to codigestion. Mesophilic AD of WAS and DAF sludge in section 6.2.4 showed codigestion of WAS and DAF sludge had better yield of biogas compared with mesophilic AD of DAF sludge of low TS, but not as good as the mesophilic AD of thickened WAS. The aim of this section is to study whether implementing TPAD can improve the biogas yield and organic removal rate.

TPAD of WAS+DAF sludge was conducted at the same time with the mesophilic AD of WAS+DAF sludge, hence, the feeding of slurry A, slurry B and slurry C was identical. The VS concentration of the feeds can be found in Figure 6-34. All the mesophilic reactors were run at a HRT of 14 days and 23 days, whereas TPAD reactors were run at first phase (55◦C) for four and six days, and then fed into second phase reactors (35◦C) for another 10 days and 17 days, respectively. By this setup, the TPAD reactors had the same total HRT as the mesophilic AD reactors. The first phase reactors fed with slurry A were operated at a HRT of four and six days (labelled as T-4-A and T-6-A), and the effluent from the first phase reactors was then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-A) and 17 days (labelled as TP-17-A), respectively.

The first phase reactors fed with slurry B were operated at a HRT of four and six days (labelled as T-4-B and T-6-B), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-B) and 17 days (labelled as TP-17-B), respectively. The first phase reactors fed with slurry C were operated at a HRT of four and six days (labelled as T-4-C and T-6-C), and the effluent from first phase reactors were then fed into the corresponding second phase reactors for another 10 days (labelled as TP-10-C) and 17 days (labelled as TP-17-C), respectively.

Likewise, on day one, reactors started with a substrate to inoculum ratio of 30:70v/v on day one and according to HRT onwards, the same as the start-up with mesophilic AD of WAS and DAF sludge. Four distinguish periods were observed similar with mesophilic AD of WAS and DAF sludge (Figure 6-35 and Figure 6-62). Period (4), which is the stable period, started around day 105 for reactors fed with slurry A, around day 95 for reactors fed with slurry B and around day 75 for reactors fed with slurry C. The results agree with the relationship between the organic content in the feed and the time reactor reaching stable, which was also found in the mesophilic AD of diluted WAS and DAF sludge. The lower the organic content in the feed, the shorter time required to stabilise the reactor.

The average daily biogas and methane from mesophilic AD and TPAD of WAS and DAF sludge are shown in Figure 6-63. The highest biogas yields of 84 to 86 mL/gVS$_{added}$ were found at reactors under a HRT of 23 days and fed with slurry C regardless of AD process tested. Comparable results were found in reactors fed with slurry B, so that the biogas yield from mesophilic AD and TPAD were almost equal. Reactors fed with slurry A, however, showed slightly less biogas yield in the TPAD process, which can be explained as the higher loading of feed caused higher amounts of nitrogenous matter in the reactors, and the TPAD enhanced the release of ammonia compared with mesophilic AD. At this point, the ammonia level reached a certain level and caused inhibitive effect on the AD process. Test results in Table 6-27 show the ammonia in T2-17-A was 6% higher than in M-23-A, and the concentration is much closer to the inhibition limit of 1600mgN/L reported by the literature.

In this experiment, that TPAD did not improve the biogas yield and it may be caused by the way reactors were started up. As mentioned, on day one, a substrate to inoculum ratio of 30:70v/v was fed into the reactors, which possibly overloaded the reactor that day. Unlike the batch test, where the organic matter was being gradually degraded in a closed environment, semi-continuous tests have organic matter being added into the system consistently. The initial high loading of organic matter can be digested over time in a batch test, whereas the change of the OL in semi-continuous conditions depends on the feeding stock in the long term when the initial inoculum is wasted and replaced by the newly grew anaerobe.

Despite this fact, the initial loading of the semi-continuous reactors effects the biogas yield in the short term (one cycle of the HRT or longer). This effect of initial loading can be extended longer than one cycle, especially when overloaded. When the reactor is overload, it creates an unhealthy or stressed environment for the microorganisms, where the growth of bacterial would be inhibited and slowed while the original number of bacterial is being wasted out. This seems to be the case here, where the overloaded start-up caused the low biogas yield. In TPAD reactors, the total HRT was the same as the mesophilic reactors; however, the individual phase reactors had HRT less than the mesophilic reactors. As the shorter HRT means a higher loading rate, individual phase TPAD reactors received an even higher loading on day one, and reactors were under more stress than mesophilic reactors at the same total HRT. Thus, the TPAD did not show advantages in biogas yield with the enhanced hydrolysis stage in first phase reactors.

The average daily biogas and methane from mesophilic AD and TPAD of codigestion of WAS and DAF sludge are shown in Figure 6-63. The highest biogas yield of 86 mL/gVS$_{added}$ was found at mesophilic AD of WAS and DAF sludge under a HRT of 23 days feeding with slurry C (M-23-C). This amount was found to be similar with T1-6-C+T2-17-C, which was fed with the same waste and run at the same total HRT. As mentioned earlier, the TPAD not having an advantage in biogas might be due to the initial overloading of the system.

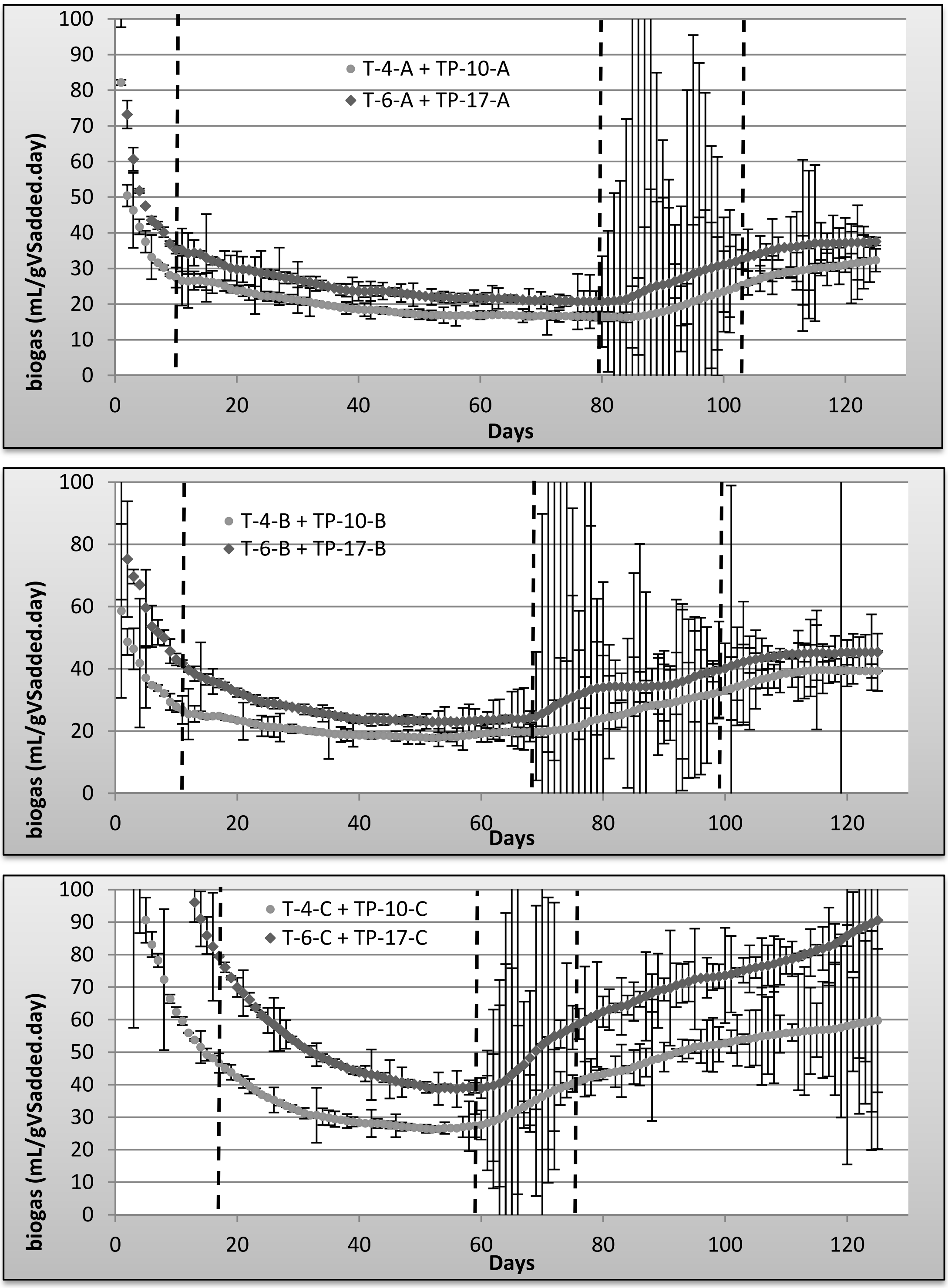

Figure 6-62: Daily biogas yield from TPAD of WAS and DAF sludge

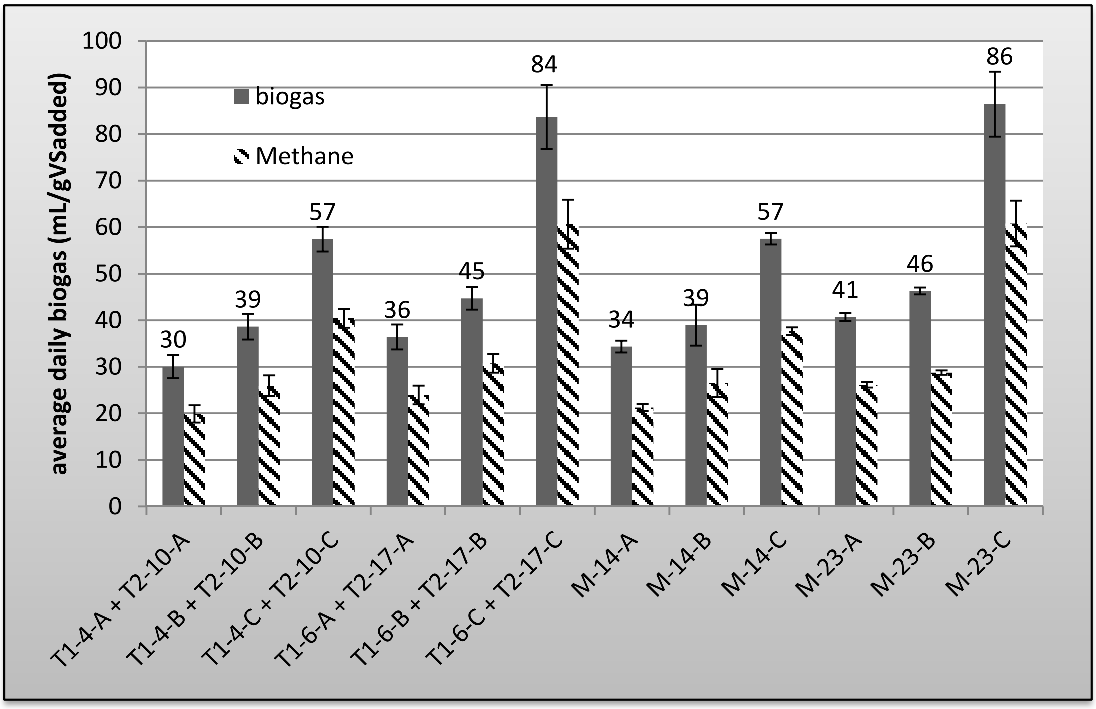

Figure 6-63: Average daily biogas yield from mesophilic AD and TPAD of WAS and DAF sludge (after day 105)

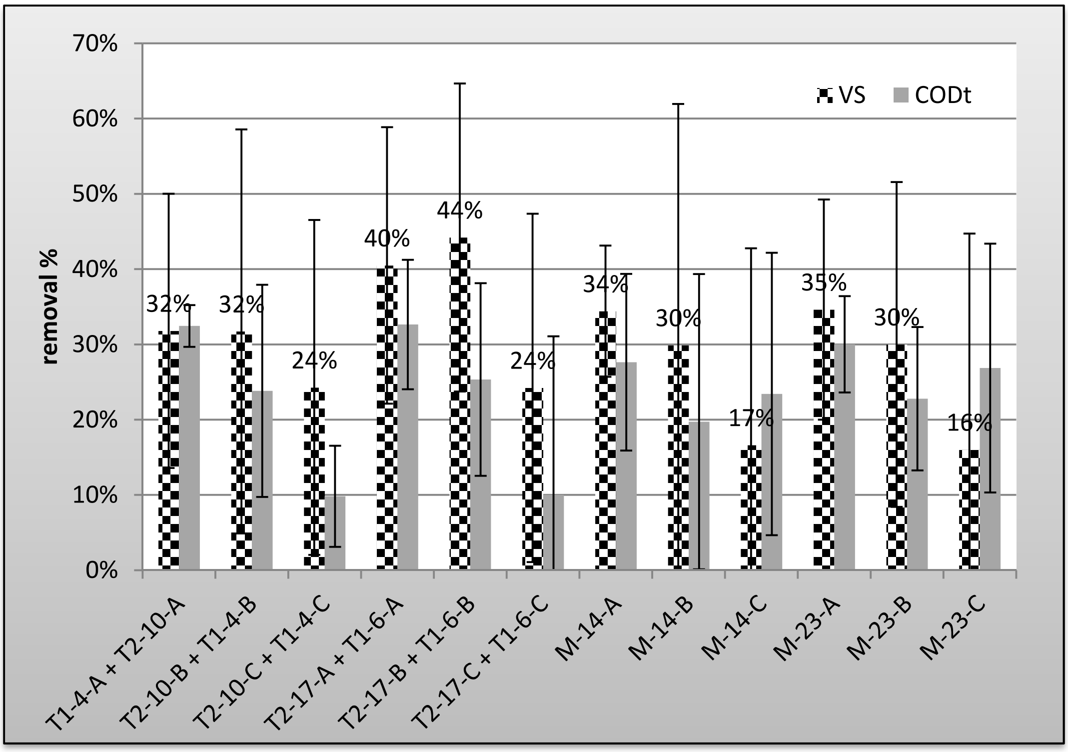

Figure 6-64: Organic removal from mesophilic AD and TPAD of WAS and DAF sludge

The average VS reduction in AD of WAS and DAF sludge ranged from 24% to 44% at various conditions under TPAD, whereas the VS reduction varied from 16% to 35% under the mesophilic AD of the same feedings (see Figure 6-64). Similar to the mesophilic AD, with the extended HRT, unlike the biogas yield, the VS reduction was not improved in TPAD reactors fed with slurry C. However, the VS removal rate did increase in TPAD reactors when using higher concentrated feeds of slurry A and slurry B.

Table 6-27 lists the parameters measuring the quality of effluent from mesophilic AD and TPAD of WAS and DAF sludge. All reactors had pH within the trade waste limits, yet only reactors fed with slurry C (the lowest organic content) had effluent to satisfy the trade waste criteria for TVFA. Though all the reactors exceeded the ammonia level, reactors fed with slurry C had the least ammonia concentration compared with other reactors, since less nitrogenous matter presented in the feed.

Table 6-27: Effluent Quality of Mesophilic AD and TPAD of WAS and DAF Sludge (Over the Stable Period)

Reactors	pH	CODs (mg/L)	TAN (mgN/L)	TVFA (mg/L)
T2-10-A	6.81±0.20	16,080 ±3167	1,551 ±443.7	3,892 ±1892
T2-10-B	6.75±0.11	12,832 ±4658	1,146 ±458.8	3,548 ±1851
T2-10-C	6.77±0.12	4,117 ±1106	374 ±38.3	987 ±259
T2-17-A	6.84±0.14	14,720 ±2560	1,493 ±229.1	3,881 ± 1180
T2-17-B	6.89±0.13	10,520 ±2177	1,305 ±638.8	2,883 ±770
T2-17-C	7.04±0.15	4,053 ±1354	502 ±161.3	859 ±391
M-14-A	6.75±0.11	15,683 ±2704	1,515 ±453.8	4,587 ±1146
M-14-B	6.75±0.08	9,953 ±2851	963 ±387.7	2,949 ±1353
M-14-C	6.69±0.09	3,150 ±793	409 ±149.2	1,085 ±787
M-23-A	6.79±0.08	14,867 ±3484	1,411 ±528.9	4,340 ±1812
M-23-B	6.74±0.19	12,243 ±1617	1,144 ±259.8	3,325 ±1196
M-23-C	6.95±0.13	3,503 ±759	457 ±95.6	872 ±208
Trade Waste Limits (CWW)	**6.00-10.00**	**4000**	**200**	**1000**

When testing settling property, effluent was settled by gravity for four hours and a photo was taken, as shown in Figure 6-65. A layer of floating scum was found similar to mesophilic AD of WAS and DAF sludge, except the T2-17-C. With higher organic content in the feed, the scum layer was thicker. The layer is likely caused by the lipids contributed by the DAF sludge, which has a tendency to form floating scum (Salminen, EA et al.,2002b). Compared with the mesophilic AD of WAS and DAF sludge, the floating scum in the effluent from the TPAD was thinner. This can be explained as the enhancement of the hydrolysis stage in the first phase reactors reducing more the lipids and degrading them into simpler organic matter. As a result, the scum layer became less.

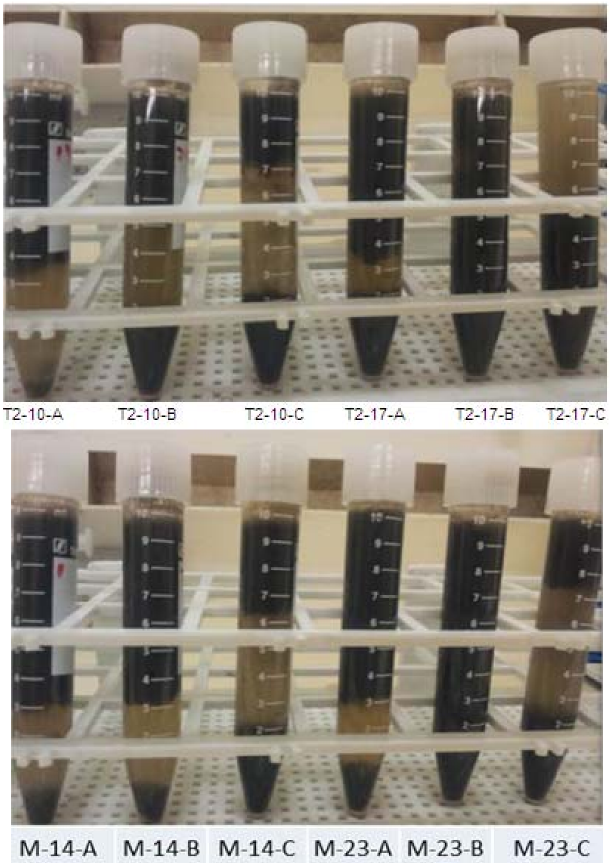

Figure 6-65: Settling condition of mesophilic AD and TPAD of WAS and DAF sludge

Table 6-28: Summary of Experimental Result from TPAD of WAS and DAF Sludge

Reactors	HRTs(day)		OLRs		Biogas	Methane	COD	VS
	First	Second	gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day		Removal (%)	
T1-4-A	4		0.75	3.85	30	20	32%	32%
T2-10-A		10	0.25					
T1-4-B	4		1.02	2.51	39	26	24%	32%
T2-10-B		10	0.30					
T1-4-C	4		0.85	1.05	57	40	10%	24%
T2-10-C		10	0.30					
T1-6-A	6		0.50	2.34	36	24	33%	40%
T2-17-A		17	0.17					
T1-6-B	6		0.75	1.53	45	31	25%	44%
T2-17-B		17	0.20					
T1-6-C	6		0.52	0.64	84	61	10%	24%
T2-17-C		17	0.19					

6.3.5. **Comparison of TPAD of Different Substrates Under Semi-continuous Conditions**

A summary of the above test results can be found in Table 6 29. The main effects plots generated from Minitab shows longer HRT and TPAD yielded higher biogas compared with lower HRT and mesophilic AD (see Figure 6 66). However, the feeding materials have more significant effects compared with the HRT and AD process.

Though OLR does not show any linear relationship with the biogas yield in Figure 6 66, when eliminating the effect from different materials, similar trends of low OLR yielding higher biogas was found with mesophilic AD (see section 6.2.5). When using the interaction plot to analyse the relationship between the HRT, process and material with biogas yield, OLR was excluded to reduce the complexity.

When separating the digest material, TPAD of WAS showed the greatest improvement in biogas yield compared with mesophilic AD within the testing conditions (see Figure 6 67). In contrast, TPAD of biosolids and TPAD of DAF sludge showed similar biogas yield with mesophilic conditions. When codigestion of WAS was with other substrate such as DAF sludge and biosolids, TPAD showed some improvement in biogas yield, which can be a result of the WAS fraction in the feeding mixture.

Among all the conditions tested, the highest methane yield was found in TPAD of WAS at HRT of 14 days, thus the highest energy recovery potential. In terms of the solids reduction, TPAD of raw DAF sludge at HRT of 28 days removed highest VS percentage of 55% though its biogas yield was as low as 34 mL/g VS.day. Hence, AD of raw DAF sludge might not be feasible for energy recovery, but could be effectively for sludge stabilization.

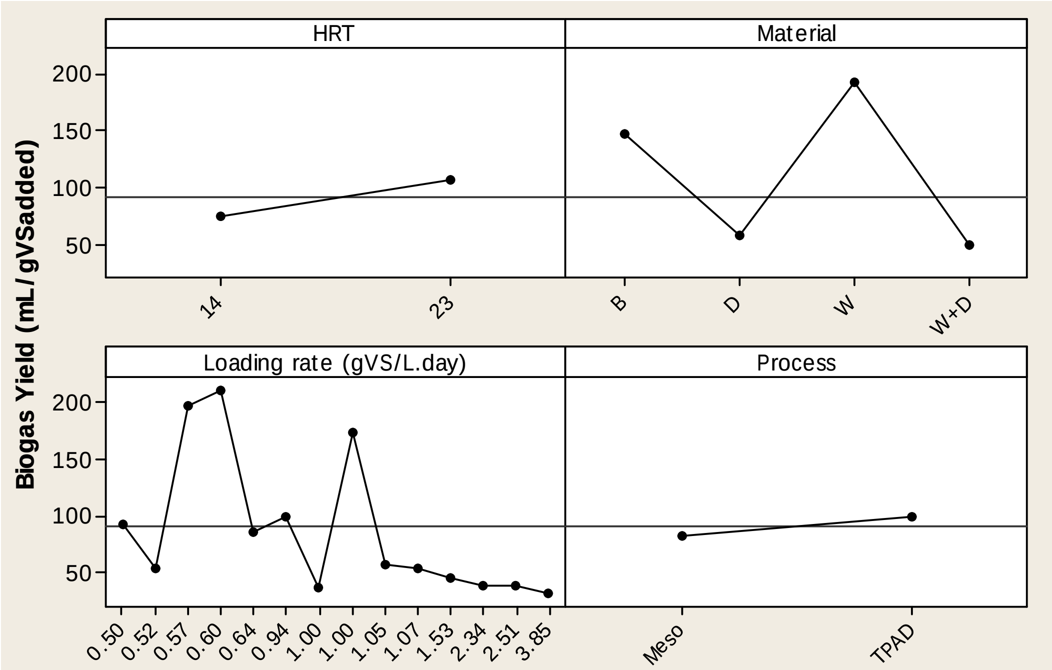

Figure 6-66: Main effect plot for biogas yield from mesophilic AD and TPAD of WAS, biosolids, DAF sludge and the combinations of WAS and DAF sludge

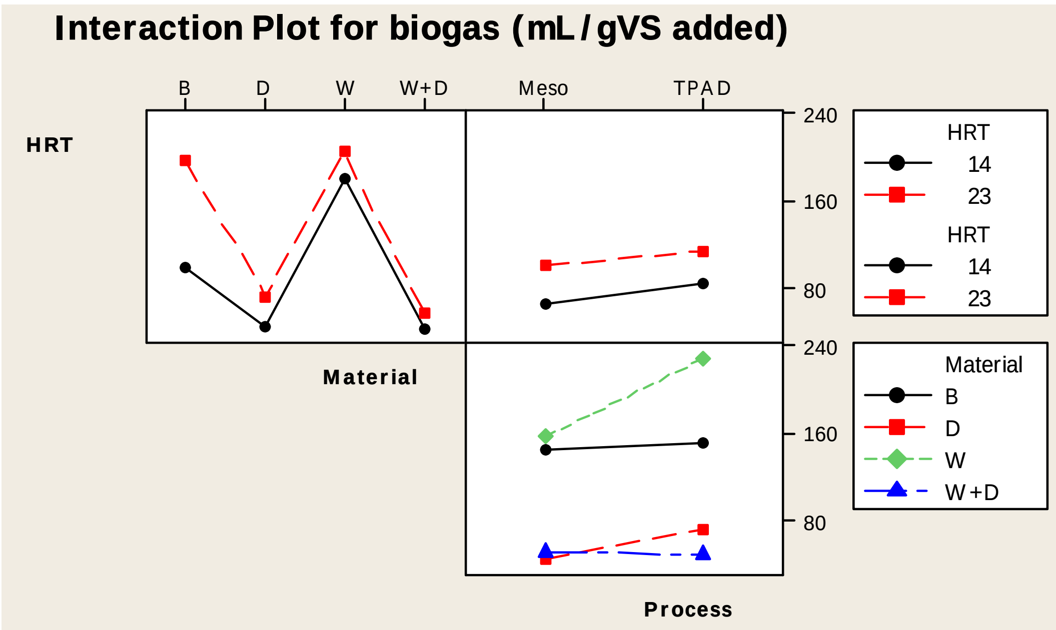

Figure 6-67: Interaction plot for biogas yield from mesophilic AD and TPAD of WAS, biosolids, DAF sludge and the combinations of those waste materials

Table 6-29: Summary of Experimental Result from TPAD of WAS and DAF Sludge

Materials	HRTs(day)		OLRs		Biogas	Methane	COD	VS	Start up
	Frist	Second	gCOD/gVS	g VS/L.day	mL/gVS$_{added}$.day*		Removal (%)		
Thickened WAS	4		0.55	0.90	235	154	26%	32%	Substrate to inoculum ratio according to HRTs on day 1 and onwards
		10	0.26						
	6		0.35	0.55	223	134	32%	33%	
		17	0.16						
Diluted biosolids	2		1.23	0.94	96	69	35%	36%	
		12	0.21						
	2		1.23	0.57	206	147	33%	29%	
		21	0.10						
Raw DAF sludge	8		0.77	3.5	22	12	33%	36%	
		15	0.25						
	8		0.77	2.9	34	18	46%	52%	
		20	0.24						
DAF sludge of low TS	4		0.69	1.07	53	36	35%	40%	
		10	0.41						
	6		0.46	0.65	93	64	41%	49%	
		17	0.30						
Non-polymer DAF sludge	4		0.78	1.01	75	53	43%	44%	
		10	0.49						
	6		0.53	0.62	79	57	42%	46%	
		17	0.29						
DAF sludge + WAS	4		0.75	3.85	30	20	32%	32%	Substrate to inoculum ratio of 30:70v/v on day 1 and ratio
		10	0.25						
	4		1.02	2.51	39	26	24%	32%	
		10	0.30						
	4		0.85	1.05	57	40	10%	24%	
		10	0.30						
	6		0.50	2.34	36	24	33%	40%	
		17	0.17						
	6		0.75	1.53	45	31	25%	44%	
		17	0.20						
	6		0.52	0.64	84	61	10%	24%	
		17	0.19						

* 100 mL/gVS = 0.1 m^3/kgVS

7. Energy Recovery

To evaluate the potential of energy recovery from the sludge produced at the ATP using AD, an energy balance around the proposed AD reactor is required in order to calculate the true energy gain. The energy required comprises the energy input for (1) maintaining the temperature and (2) mixing the contents inside the reactor. The energy output is that recovered through methane gas. In addition, loss of energy due to leakage or inefficient insulation is included. Energy consumption for sludge management at ATP using aerobic digestion (i.e., current practice compared with proposed AD treatment) is discussed below.

7.1 Energy Input for AD Treatment

The energy input for the proposed AD system includes the transportation of raw materials and digestate, and operational requirement (heating and mixing).

7.1.1. **Transportation**

When comprises the cost transportation of raw materials for the AD system, the most energy efficient way is to treat the sludge onsite to reduce the cost of the transportation of raw materials. However, when codigestion involves more than one substrate, for example, WAS and DAF sludge, ideally a small amount or weight of the substrate should be taken to the site of where other substrates are generated.

For instance, when codigestion of DAF sludge and WAS is at the ratio of 10:90 v/v (experiment discussed in section 6.2.4), DAF sludge should be transported to ATP to minimize the energy associated with transportation. In this case, 0.14 ML/day of DAF sludge would be transported from the meat industry to ATP. If a truck is the method of transportation, energy input is 1.6 ML/ton.km (Berglund et al.,2006). (Berglund et al.,2006). The distance between the two facilities is around 10 km. Assuming DAF-sludge has specific gravity of 1.05 , the energy input for transportation is::

$$Input_{transportation} = \frac{1.6 \times 10^6 J}{km.ton} \times 10km \times \left(\frac{0.14 \times 10^6 L}{day} \times \frac{1.05 \times 1ton}{1000L}\right)$$
$$= 2.352E + 09\ J/day$$

7.1.2. **Heating Requirement**

This represents the energy required to raise the temperature of the incoming sludge temperature to the designated temperature (Zupančič et al.,2003) as well account for the heat losses. Annual average temperature in the Altona region is 19.7°C (BOM,2013), which is the mean maximum monthly data.

7.1.2.1. ***Heat Loss***

Heat losses from the digester contribute the heat loss from sludge to air, loss from sludge to soil and loss from sludge to ground water. Those losses are proportional to the contact surface area of the digester. With a longer HRTs, the digester has a larger volume, and bigger surface area, hence, the heat loss is greater. The heat loss can be calculated as below(Metcalf et al.,2003):

$$\boldsymbol{q} = \sum(\boldsymbol{U} \times \boldsymbol{A} \times \Delta\boldsymbol{T}) \qquad \textbf{Equation 7-1}$$

Where,

q = eat loss, J/s,

U = overall coefficient of heat transfer, W/m2.°C

A = contact surface area, m2

ΔT = the temperature drop across the surface, °C

Table 7-1: Overall Heat Coefficient

Overall Coefficient of Heat Transfer	Air	Soli	Ground Water
W/m^2.°C	0.91	0.68	0.85

The design of the digester shape determines the surface contact area with air, soil and ground water (see Figure 7-1). The diameters (d), side depth (h_1) and mid-depth (h_2) are based on the required volume of the digester (10% v/v head space required). Calculations of the surface areas based on geometric formula shows in Table 7-2.

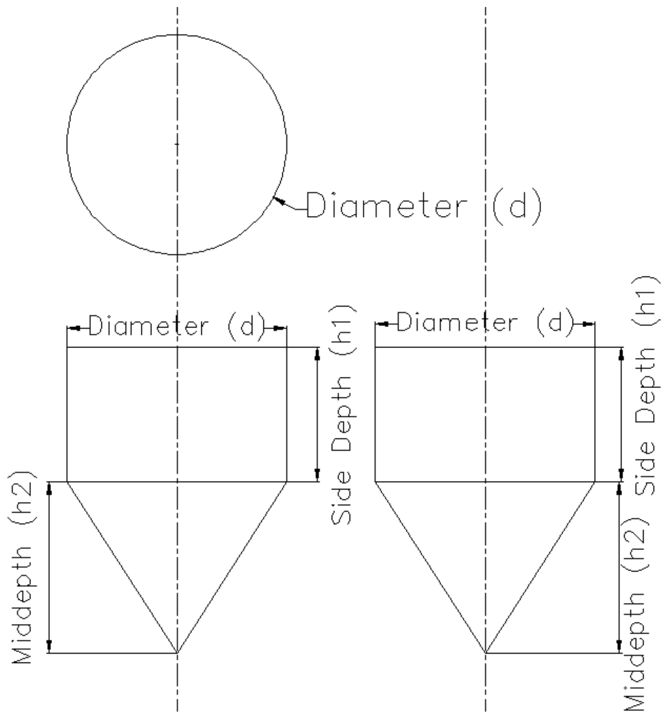

Figure 7-1: Shape of an AD digester

Table 7-2: Equations for Calculations of Digester Surface Area

	Equations
Roof area (contact with air)	$A_{roof} = \frac{\pi \times d^2}{4}$
Wall area (contact with Soli)	$A_{wall} = \pi \times d \times \ (h_1)$
Floor area (contact with ground water)	$A_{floor} = \pi \times \ (\frac{d}{2}) \times \sqrt{(h_2)^2 + \ (\frac{d}{2})^2}$

Annual average temperature in Altona region is 19.7°C C (BOM,2013). The sludge and groundwater temperature are estimated according to the empirical relationship between ambient temperature and the temperatures of sludge and groundwater. Thus the sludge and groundwater temperature is calculated as 22.2°C. This empirical relationship is (Monteith et al.,2012):

$$\boldsymbol{Fluid\ temperature} = \mathbf{0.607} \times \boldsymbol{Ambient\ temperature}(℃) + \mathbf{10.2}(℃) \quad \textbf{Equation 7-2}$$

7.1.2.2. *Heating Incoming Sludge*

For a mesophilic AD system, sludge of 22.2℃ is required to be heat up to 35°C, and in TPAD systems, sludge needs to be heated up to 55 °C.

Energy consumption for heating sludge temperature to 35 degrees can be estimated by considering the sludge heat capacity as equal to water, thus the heat capacity of sludge is 4.184 kJ/kg.°C (Yang et al.,2010).

The heating for incoming sludge to mesophilic digester is then given as:

$$\boldsymbol{q} = (\mathbf{35}℃ - \mathbf{22.2}℃) \times \mathbf{4184}\,\frac{\boldsymbol{J}}{\boldsymbol{kg}.℃} \times \boldsymbol{weight\ of\ sludge}(\boldsymbol{kg}) \qquad \textbf{Equation 7-3}$$

For example, if the codigestion of DAF sludge and WAS is at the total flow rate of 1.44ML/day and HRT of 23 days, the heating requirement is 7.76E+10 J:

$$q = (35℃ - 22.2℃) \times 4184\,\frac{J}{kg.℃} \times \frac{1.44\times10^{6}L}{day} \times \frac{1kg}{1L} = 7.76E + 10\ J/\text{day}$$

7.1.3. **Mixing**

Not only goes heating the sludge consume energy, mixing and pumping also requires electricity. However its consumption depends on the plant configuration and mixing method.

Generally, the AD process requires mixing in order to fully distribute the heat and minimise the dead space inside the reactor. There are different mixing types, such as mechanic mixing and gas mixing. However, gas mixing is widely used in the industry as it does not require moving parts that the energy requirement is minimal. The US EPA (1979) recommends an energy input range of 5 to 8 W/m^3 for a proper gas mixed anaerobic system.

Thus, for the codigestion of DAF sludge and WAS (10:90 v/v) at the total flow rate of 1.44ML/day and HRT of 23 days, the energy input for mixing is , the calculation is shown below:

$$q = \frac{1.44 \times \frac{10^{6}L}{day} \times 23\ day}{day} \times \frac{5W}{1000L} \times \frac{1\ J/s}{1\ W} \times \frac{86400\ s}{1\ day} = 1.44E + 10\ J/day$$

7.2 Output

Energy generation from AD is by the recovery of methane gas. Methane gas has a net heating energy content as 35800 kJ/m^3 STP (Metcalf et al.,2003). The amount of recovered energy can be calculated as electricity generation from the methane.

For example, when codigestion of DAF sludge and WAS at the ratio of 10:90 v/v (experiment discussed in section 6.2.4), the average VS of the feed was 17,660mg/L, the daily feeding flow rate was 1.44ML/day, and the methane yield was 64 mL/gVS. Thus, the daily methane yield can calculate as follow:

$$Daily\ methane\ yield\ = \frac{1.44 \times 10^6 L}{day} \times \frac{17.66\ gVS}{L} \times \frac{64mL\ CH_4}{gVS} \times \frac{1m^3}{10^6 mL} = 1627\ m^3\ CH_4/\ d$$

To correct the temperature from 35 degree:

$$Daily\ methane\ yield\ = (\frac{273K + 35K}{273K})\ \times\ 1627\ m^3\ CH_4/\ d\ =\ 1836\ m^3\ CH_4/\ d$$

The energy output is:

$$\frac{1836\ m^3 CH_4}{day} \times \frac{35800kJ}{m^3} \times \frac{1000J}{kJ} = 6.57E + 10\ \ J/d$$

7.3 Case Studies

Results from some tested conditions in section 6 are applied in Table 7-3. Their data are used as case studies of the energy recovery rate. The selection of the test conditions are based on the high methane yield and low HRTs, listed below:

(1). Mesophilic AD of ATP thickened WAS under a HRT of 23 days,

(2). TPAD of ATP thickened WAS under a HRT of 4 + 10 days,

(3). Mesophilic AD of ATP Biosolids under a HRT of 23 days, (i.e., solids remaining after aerobic digestion, hence energy used includes aerobic treatment of WAS).

(4). Mesophilic codigestion of ATP WAS + DAF-sludge (10:90 v/v) under HRT of 23 days

The daily flow rate of thickened WAS and biosolids was used for energy balance calculations. For example, ATP daily WAS production of 1.3 ML/day is thickened to 0.48 ML/day. The biogas and methane production from AD treatment for the above scenarios was the average methane yield measured over the period of operation of the reactors at steady state conditions (experiments discussed in Chapter 6), at the specified HRT. Table 7-3 shows, for each scenario, the AD reactors size and the estimated energy input and output. Typical energy requirement for aerobic sludge treatment is 20 to 40 W/m^3 (Metcalf & Eddy 2003). Currently, WAS enters the aerobic sludge tank and the decanted water is sent back to the head of the IDEA tank (see Figure 2-2). The thickened sludge is aerated for 18 days. The energy balance for proposed AD treatment was calculated assuming they received thickened WAS of the same characteristics of the thickened WAS that was fed into the aerobic sludge, and assuming that the daily sludge flow rate is the same. The energy recovery for the different scenarios are summarised in Table 7-3.

Comparing the energy balance for the different AD treatment scenarios and the current aerobic sludge treatment at ATP, the highest energy recovery is from mesophilic AD of biosolids (see Figure 7-2). The energy to be recovered at this condition is estimated to be 4590 MJ/day, which is equivalent to 465 MWh/year.

Though the TPAD of WAS produced higher energy output than the mesophilic AD of WAS, due to the higher operational temperature in its first phase reactor, the energy balance showed negative value, whereas the mesophilic AD of WAS could provide 100 MJ/day (or 10 MWh/yr). Similarly, for the codigestion of DAF sludge and WAS, although the energy output was the highest among the scenarios discussed, the overall energy balance showed negative results due to the energy input associated with the transportation of the DAF sludge to the site.

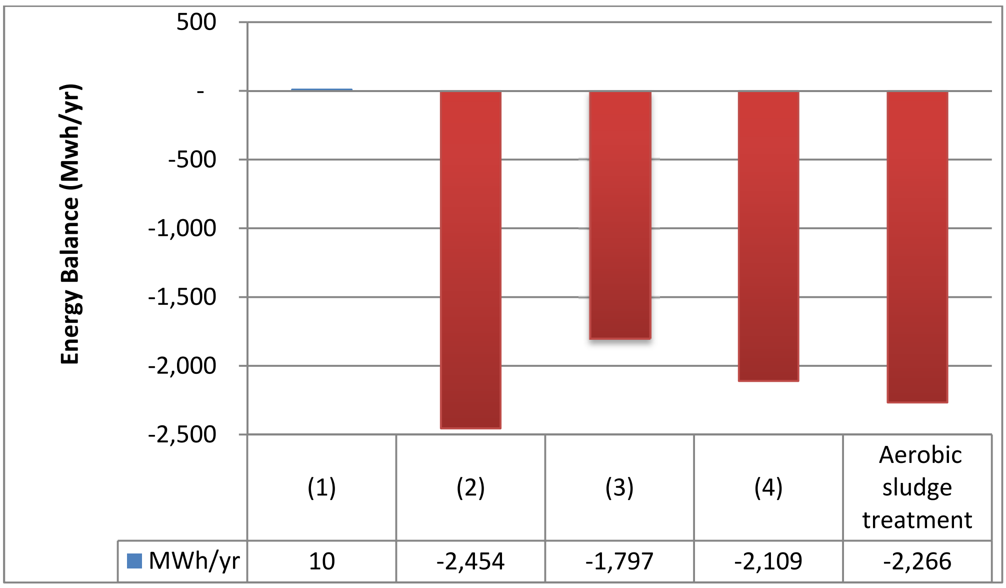

Figure 7-2: Comparison of energy balance for the different ATP sludge AD treatment scenarios

Overall, the energy recovery rate is not only the energy generated from the waste. It also takes account of the energy input for the digester operation and energy saving compared with current aerobic sludge treatment. In the light of this, the highest energy recovery option is mesophilic AD of biosolids in ATP, which can save 2,266 MWh/yr if replacing the current aerobic system, and can also generate 465 MWh/yr from its methane yield.

Table 7-3: Case Studies on Energy Balance

Testing conditions	(1)**	(2)		(3)	(4)	Aerobic Sludge Treatment*
Daily flow rate (ML/day)	0.48	0.48		0.13	1.44	0.48
HRTs (day)	23	4+10		23	23	18
VS of feed (mg/L)	15,200	15,200		17,570	17,660	15,200
Methane yield*** (mL/gVS)	113	154		136	64	-
Reactor size:		1st phase	2nd phase			
Total volume (m^3)	12,315	2,121	5,344	3,478	37,202	
- Diameters (m)	28	15	18	18	35	
- Side depth (m)	15	9	16	11	30	
- Mid depth(m)	15	9	15	8	26	
Transport (J/d)	0	0		0	2.31E+09	
heat loss(J/d) - loss to air (J/d)	7.41E+08	7.97E+08		3.06E+08	1.16E+09	
- loss to soil (J/d)	1.19E+09	1.69E+09		5.59E+08	2.97E+09	
- loss to groundwater (J/d)	7.06E+08	1.13E+09		2.50E+08	1.78E+09	
heating for income sludge(J/d)	2.57E+10	6.58E+10		7.16E+09	7.76E+10	
Mixing(J/d)	4.76E+09	2.90E+09		1.32E+09	1.44E+10	
Aeration (J/d)	0.00E+00	0.00E+00		2.23E+10	0.00E+00	2.23E+10
Total energy input(J/d)	**3.31E+10**	**6.94E+10**		**3.06E+10**	**8.58E+10**	**2.23E+10**
Energy output by methane (J/d)	**3.32E+10**	**4.53E+10**		**1.29E+10**	**6.57E+10**	**-**
Energy balance (J/day)	1.00E+08	-2.42E+10		-1.77E+10	-2.08E+10	-2.23E+10
(MWh/yr)	10	-2,454		-1,770	-2,109	-2,266

* Typical value of energy requirement for aerobic sludge treatment is assumed as of 30 W/m^3. The current aerobic sludge tank in ATP runs for retention time of 18 days. (Metcalf & Eddy 2003)

**Cases are: 1)Mesophilic AD of thickened WAS under HRT of 23 days; 2)TPAD of thickened WAS under HRT of 4+10 days; 3)Mesophilic AD of Biosolids under HRT of 23 days; 4)Mesophilic codigestion of WAS +DAF sludge(10:90 v/v) under HRT of 23 days

***data obtained from section 6

8. Conclusions

Due to the increasing energy prices, wastes rich in organics such as sludge from wastewater treatment plants are no longer considered a disposal problem, rather a substrate for energy recovery. The literature review carried out at the early stages of this study showed that AD is the most feasible option among the other energy recovery technologies, such as pyrolysis, gasification and incineration.

In terms of types of AD processes, TPAD of WAS resulted in higher biogas yield compared to one phase mesophilic AD. Similarly, TPAD also had a higher biogas yield for the codigestion of WAS with other substrates such as DAF sludge and biosolids, compared to one phase mesophilic AD. It was found that TPAD at a HRT of 23 days had a higher biogas yield compared with lower HRT and mesophilic AD. For instance, biogas yields of 223 mL/gVS from TPAD of WAS at a HRT of 23 days were higher that the biogas yields of 125 mL/gVS from AD of WAS at a HRT of 14 days.

Conversely, TPAD and mesophilic AD had a similar biogas yield when used for biosolids and DAF sludge treatment. Under these conditions, the positive effect of separating the AD phases was negated by the inhibitory effect of high concentrations of TVFA produced in the thermophilic phase (first phase of the TPAD). The DAF sludge used in these experiments was found to be low in pH, high in TVFAs and TN.

However, the waste materials were found to be the most significant factor compared with the HRT and AD process. Biogas yields for WAS were found to be 184 mL/gVS, 188 mL/gVS and 223 mL/gVS from the mesophilic batch test, semi-continuous mesophilic AD and semi-continuous TPAD, respectively. Biogas yields for DAF were found to be 215 mL/gVS, 22 mL/gVS and 34 mL/gVS from the mesophilic batch test, semi-continuous mesophilic AD and semi-continuous TPAD, respectively. Due to the characteristics of DAF sludge (high in TVFA and TN), the semi-continuous test results were much lower compared with the batch test results.

For research purposes, DAF sludge was then mixed with water to form a low TS DAF sludge. Biogas yields for DAF with low TS were found to be 398 mL/gVS, 54 mL/gVS and 64 mL/gVS for the mesophilic batch test, semi-continuous mesophilic AD and semi-continuous TPAD, respectively. Codigestion of WAS and DAF was also studied. The biogas yields for WAS and DAF were found to be 61 mL/gVS and 84 mL/gVS from semi-continuous mesophilic AD and semi-continuous TPAD, respectively (batch results were not available). The semi-continuous results were found much lower than the batch results, which indicated the continuous reactors were inhibited due to a high level of TVFA occurring.

In terms of VS reduction, TPAD of WAS and TPAD of biosolids had higher removal rate comparing with Mesophlic AD of the substrate. For instance, TPAD of WAS removed 33% of VS in its effluent whereas the removal rate at mesophilic AD of WAS was 18%. On the other hand, codigestion of DAF+WAS only showed higher VS removal rate under higher HRTs in TPAD conditions in comparison of mesophilic condition. OLR also affected the VS reduction rate in AD of DAF, as reactors with lower OLRs were found higher VS reduction. For example, mesophilic AD of DAF sludge of low TS had the VS removal increased from 27% to 40% when the reactor loadings decreased from 3.07 g VS/L.day to 0.52 g VS/L.day.

When considering energy recovery, the energy input for maintaining the temperature and mixing in the reactors were included in the calculation of the true energy gain. Energy recovery from the AD of ATP WAS, ATP WAS co-digestion with meat wastes using either conventional AD or TPAD was estimated and compared with energy usage for the ATP current aerobic sludge system. The results showed that the highest energy recovery would be using a mesophilic AD of ATP thickened WAS, onsite at ATP. Although, using this system, the energy generated from methane yield is only 10 MWh/yr, energy savings of up to 2,266 MWh/yr through phasing out of the current aerobic sludge treatment should be taken into account, which increase the viability of energy recovery from ATP WAS.

Noteworthy that the biosolids were not adequately stabilized that the BMP test of the biosolids showed its methane potential, thus existence of biodegradable organic matters. This could indicate the current aerobic sludge treatment in ATP might not achieve the designed capacity of sludge stabilization.

Based on the results of this study, WAS from ATP is feasible for energy recovery. However, there is still a potential for future research on biogas enhancement by examining pre-treatment and codigestion with other waste such as food waste or sugar waste.

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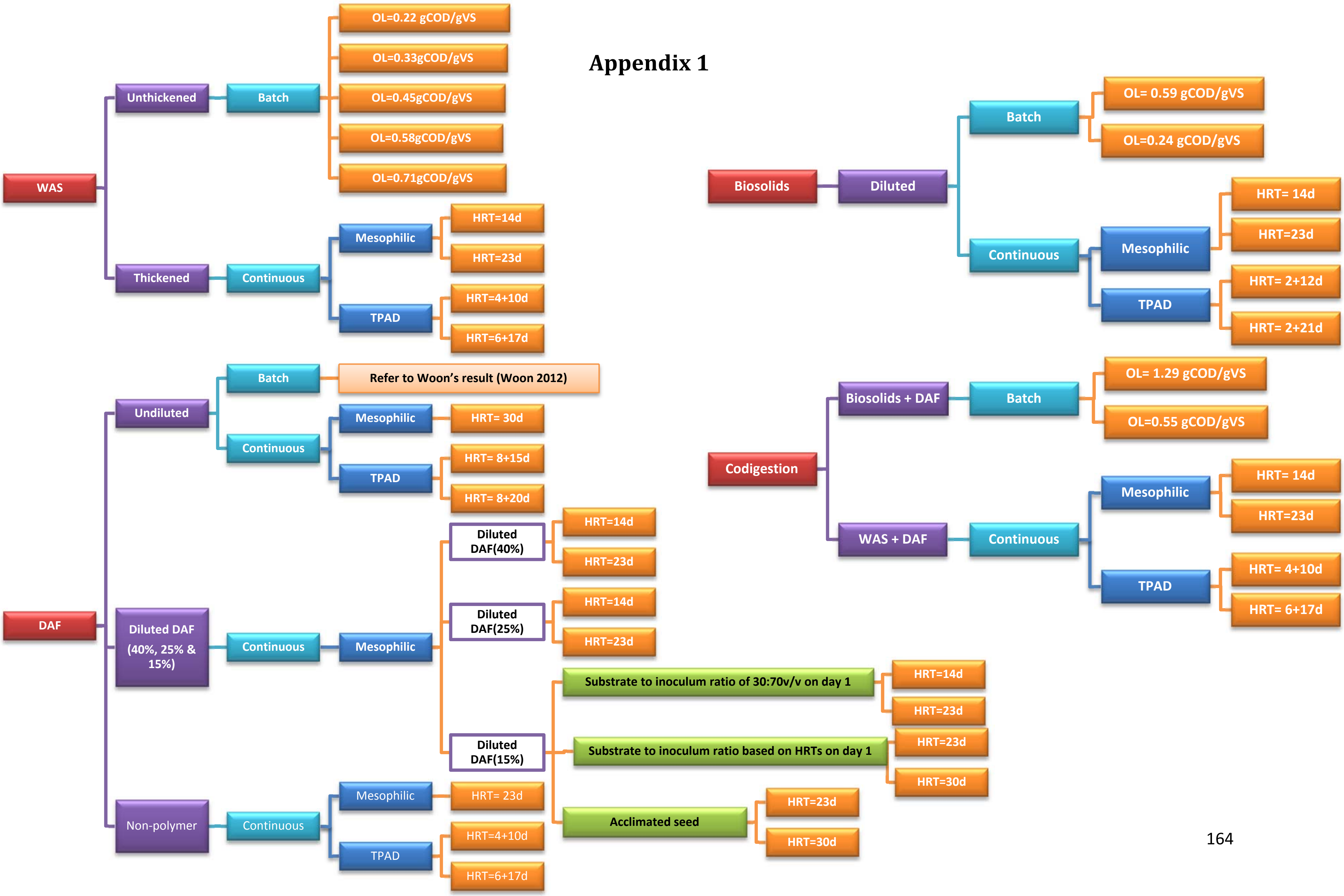
Appendix 1



WAS
Unthickened
Batch
OL=0.22 gCOD/gVS
OL=0.33gCOD/gVS
OL=0.45gCOD/gVS
OL=0.58gCOD/gVS
OL=0.71gCOD/gVS
Thickened
Continuous
Mesophilic
HRT=14d
HRT=23d
TPAD
HRT=4+10d
HRT=6+17d
DAF
Undiluted
Batch
Refer to Woon's result (Woon 2012)
Continuous
Mesophilic
HRT= 30d
TPAD
HRT= 8+15d
HRT= 8+20d
Diluted DAF
(40%, 25% & 15%)
Continuous
Mesophilic
Diluted DAF(40%)
HRT=14d
HRT=23d
Diluted DAF(25%)
HRT=14d
HRT=23d
Diluted DAF(15%)
Substrate to inoculum ratio of 30:70v/v on day 1
HRT=14d
HRT=23d
Substrate to inoculum ratio based on HRTs on day 1
HRT=23d
HRT=30d
Acclimated seed
HRT=23d
HRT=30d
Non-polymer
Continuous
Mesophilic
HRT= 23d
TPAD
HRT=4+10d
HRT=6+17d
Biosolids
Diluted
Batch
OL= 0.59 gCOD/gVS
OL=0.24 gCOD/gVS
Continuous
Mesophilic
HRT= 14d
HRT=23d
TPAD
HRT= 2+12d
HRT= 2+21d
Codigestion
Biosolids + DAF
Batch
OL= 1.29 gCOD/gVS
OL=0.55 gCOD/gVS
WAS + DAF
Continuous
Mesophilic
HRT= 14d
HRT=23d
TPAD
HRT= 4+10d
HRT= 6+17d

Appendix 2

Treatment Grades Based On Treatment Process, Microbiological Criteria And Other Suggested Controls(EPA Victoria,2004)

Treatment process	Associated controls
Treatment Grade T1: Very low pathogen levels with minimum regrowth potential	
Composting processes that simultaneously heat all material (e.g. in-vessel) Temperature of all compost material to be maintained at ≥55°C for ≥3 continuous days.	Relevant vector attraction reduction controls and production of product that does not generate offensive odours. Weed seed controls may be needed in landscaping or agricultural applications.
Composting windrow method Temperature of compost material maintained at ≥55°C for ≥15 days, including 5 turnings of the windrow.	
High pH and high temperatures Biosolids pH raised to ≥12 for ≥72 continuous hours and during this period, maintained at ≥52°C for ≥12 continuous hours. Final biosolids product to be air-dried to a solids content of ≥50%.	Relevant vector attraction reduction controls (refer Table 4) and production of product that does not generate offensive odours.
Heating and drying Biosolids dried by heating particles to ≥80°C to a final solids content of ≥90%.	
Thermophilic digestion processes Processes operating at greater than 55°C will be considered on a case-by-case basis depending on retention time, process stages and batch versus continuous feed/draw.	
Long-term storage Sludge is digested, dewatered to >10% w/w solids and stored for > 3 years.	Product must be stored in manner that ensures no recontamination and not generate offensive odours.
Treatment Grade T2low pathogen levels but some pathogen regrowth potential	
Composting method The temperature of all compost material to be ≥53°C for ≥5 continuous days or ≥55°C for ≥3 continuous days. (NB. Although this criteria is comparable to T1, it is also included as a T2 process in reflection that achieving the stringent T1 E.coli limits may require specialised techniques.	Relevant vector attraction reduction controls (e.g. ≥38% VS reduction in corporation into soil within 6 hours). Weed seed controls may be needed in landscaping or agricultural applications.
Heating and drying Biosolids are heated to ≥70°C and dried to a solids content of at least 75% w/w.	Relevant vector attraction reduction controls and product that, coupled with management controls, does not generate offensive odours.
Aerobic thermophilic digestion Aerobic conditions at 55-60°C for ≥10 continuous days. Final product dried to ≥50% solids. (NB. Could also achieve T1 process).	
Treatment Grade T3 established processes with pathogen reduction	
Anaerobic digestion ≥15 days at ≥35°C or ≥60 days at ≥15°C.	Relevant vector attraction reduction controls and product that, coupled with management controls, does not generate offensive odours. Weed seed controls may be needed in landscaping or agricultural applications.
Aerobic digestion ≥40 days at ≥20°C or ≥60 days at ≥15°C.	
Composting Aerobic conditions maintained ≥5 days at ≥40°C including ≥4 hours at ≥55°C.	

Appendix 3

Biosolids Classification and Permissible End Uses (EPA Victoria,2004)

	Sludge Grade		
Treatment Grade	C1	C2	Worse than C2
T1	Unrestricted Uses, Restricted Uses (1,2,3,4,5,6)	Restricted Uses (1,2,3,4,5,6)	Not consider for sustainable uses on land applications
T2	Restricted Uses (2,3,4,5,6)	Restricted Uses (2,3,4,5,6)	
T3	Restricted Uses (3,4,6)	Restricted Uses (3,4,6)	
Worse than T3			

Unrestricted Uses	Biosolids are suitable for distribution, marketing and appropriate use with only minimal controls (e.g. recommended handling/safety directions). Includes sale as a bagged product for residential use.		
Restricted Uses	*Agricultural Uses*	1	Human food crops consumed raw in direct contact with biosolids,
		2	Dairy and cattle grazing/fodder (also poultry), human food crops consumed raw but not in direct contact
		3	Processed food crops
		4	Sheep grazing and fodder (also horses, goats), on food crops, woodlots
	Non-Agricultural Uses	5	Landscaping (unrestricted public access)
		6	Landscaping, (restricted public access), forestry, land rehabilitation